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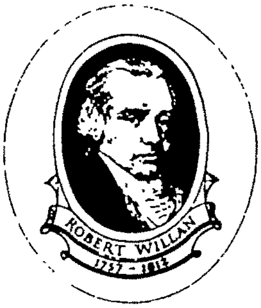

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T4/T8 ratio and absolute T4 cell numbers in different clinical stages of Kaposi's sarcoma in AIDS

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SUMMARY

Thirty-seven men (36 homosexual or bisexual and one heterosexual) with epidemic Kaposi's sarcoma and underlying HIV infection were followed up over a period of up to 32 months. Fourteen patients (38%) died, with a median survival time of 7·2 months after the diagnosis of AIDS. Seventeen patients (46%) presented with one or more opportunistic infections, mostly *Pneumocystis carinii* pneumonia. Eighteen patients (49%) had lymphadenopathy syndrome according to the definition of the CDC. Using the Laubenstein-classification of Kaposi's sarcoma, all patients either remained stable or deteriorated, improvement was never observed. Absolute T4 lymphocyte counts and the T4/T8 ratio were not related to the disease stage. With the onset of B symptoms (systemic symptoms), however, the absolute T4 numbers and the T4/T8 ratio markedly decreased. Delayed type hypersensitivity also showed no relationship to the clinical stages of Kaposi's sarcoma. Thus, the clinical progression of Kaposi's sarcoma lesions seems to be largely independent of the immunological parameters investigated. However, the onset of B symptoms was observed to be related to changes in immune status.

Disseminated Kaposi's sarcoma (epidemic type) is one of the main manifestations of the acquired immune deficiency syndrome (AIDS) and occurs almost exclusively among homosexual men.[1–3] Patients with epidemic and also with classical Kaposi's sarcoma seem to have a greater susceptibility to other malignancies including non-Hodgkin's lymphomas and leukaemias,[2,4,5] pointing to a possible genetic factor.

The appearance of Kaposi's sarcoma has also been linked to infections with several viruses, especially cytomegalovirus and Epstein-Barr virus which are known to be capable of transforming lymphocytes *in vitro*.[4,6,7] Cytomegalovirus infection is also known to cause immunosuppression in both humans and mice.[8]

Furthermore, it has been observed that patients receiving immunosuppressive therapy, for example renal transplant recipients, showed an increased incidence of epidemic Kaposi's sarcoma.[2,4] These findings suggest a possible relationship between immunosuppression and the onset of this otherwise unusual tumour. AIDS patients with Kaposi's sarcoma only, compared with those with Kaposi's sarcoma plus opportunistic infections, present with a better clinical and immunological status and have a greater life expectancy.[4,9] Considering the different clinical stages of Kaposi's sarcoma, we hypothesized that with progression of the disease the immunological parameters would be expected to continue to deteriorate.

We have studied T4 and T8 cells and delayed type hypersensitivity in patients with different clinical stages of Kaposi's sarcoma.

METHODS

Patients

Thirty-six Caucasian homosexual or bisexual men, and one heterosexual man, with epidemic Kaposi's sarcoma and underlying HIV infection were examined. The mean age was 38 years (range 25–65 years). The mean observation time was 8·1 months and ranged from 1 to 32 months (up to the end of February 1987).

Diagnosis of Kaposi's sarcoma was confirmed by biopsy. The onset of AIDS (if the patient did not show any previous opportunistic infections) was determined by the date of the first biopsy revealing Kaposi's sarcoma, even if the patient had given a history of purple blue lesions before this. Diagnosis of opportunistic infections according to the definition of the Centers for Disease Control (CDC), Atlanta, U.S.A. was proven by bronchoscopy and bronchial lavage (*Pneumocystis carinii* pneumonia), oesophago-gastro-duodenoscopy (*Candida* oesophagitis) and by electron microscopy using a negative contrast technique (ulcerating herpes simplex). If this latter procedure failed to show virus-like particles the diagnosis was made clinically on typical morphological features. Toxoplasmosis and disseminated cytomegalovirus infection observed in one patient were diagnosed at autopsy.

Clinical staging of Kaposi's sarcoma

The clinical stages of Kaposi's sarcoma were determined using the classification of Krigel, Laubenstein and Muggia[10] with slight modifications: Grade I cutaneous, locally indolent; Grade II cutaneous, locally aggressive with or without palpable regional lymph nodes; Grade III mucocutaneous with or without palpable regional lymph nodes; Grade IV visceral; A no systemic signs or symptoms; B systemic signs: nightsweats, fever, weight loss of more than 10%.

Human immunodeficiency virus (HIV) serology

As a screening test, an enzyme-linked immunosorbent assay (ELISA) was used (Du Pont de Nemours, Bad Nauheim, Germany). All results were confirmed by Western blot or immunofluorescence or both.

Immunological parameters

Lymphocyte subpopulations. Peripheral blood mononuclear cells were isolated from heparinized blood by Ficoll-Hypaque density gradient separation and cells were stored in liquid nitrogen. For immunofluorescence, cells were reacted with monoclonal antibodies of the CD4 cluster (helper cells, MT 151,)[11] and of the CD8 cluster (cytototoxic/suppressor cells, MT

811,)[11] at saturating concentrations for 30 min on ice. After two washes the cells were incubated with goat anti-mouse Ig FITC (Tago, Burlingame, California, U.S.A.) for another 30 min and after final washes they were analysed under a Leitz orthoplan fluorescence microscope with PLoem epi-illumination. In each sample 150–200 cells were analysed. The percentage of positive cells was used to calculate the T4/T8 ratio and the absolute counts of lymphocytes and monocytes in the peripheral blood. The ratio of T helper to T suppressor/cytotoxic cells and the absolute numbers of T helper cells were determined in 29 and 24 patients, respectively. In most patients several examinations were carried out during the course of the study.

Delayed type hypersensitivity. For evaluation of cellular immunity an intradermal test with recall antigens was performed using the 'Multitest Merieux' system providing seven microbial antigens and one control (glycerol). The tests were read after 48 h and the diameters of the positive skin reactions measured. The patients were divided into three categories on the basis of the sum of the diameters of the skin test reactions: Anergy (0–1 mm), hypoergy (2–9 mm), normoergy (> 10 mm).

Statistical analysis

The groups were compared using a Mann Whitney U test.

RESULTS

Clinical data

All patients fulfilled the clinical criteria of the CDC for AIDS, together with positive HIV serology including confirmatory tests.

Seventeen of the 37 patients (46%) presented with one or more opportunistic infections either before or after the diagnosis of Kaposi's sarcoma. Most developed *Pneumocystis carinii* pneumonia and presented at the same time or later with ulcerating herpes simplex infection and *Candida* oesophagitis. Additionally, many patients showed various skin infections including herpes zoster infection, non-ulcerating herpes simplex, molluscum contagiosum and multiple infections with human papilloma viruses such as verrucae vulgares or condylomata acuminata. Oral, genital and intestinal candidosis was also detected (Table 1). Eighteen patients (49%) showed signs of lymphadenopathy syndrome according to the definition of the CDC, together with Kaposi's sarcoma. Almost all patients (35) had a past history of other sexually transmitted diseases including syphilis, gonorrhoea and hepatitis B.

During the period of observation 14 patients died (38%); seven of these had developed opportunistic infections. In four cases the cause of death remained unclear because autopsy was refused. One patient committed suicide after the diagnosis of *Pneumocystis carinii* pneumonia was made and another, a renal transplant recipient (HIV-positive, homosexual), died of acute renal failure. In three patients death seemed to be directly caused by Kaposi's sarcoma; two died with gastrointestinal obstruction due to Kaposi's sarcoma and one patient died of obstructive, extremely exophytic lesions of the tumour in the pharynx and larynx. The average time of survival after the diagnosis of AIDS was 7·2 months.

All patients who later died had suffered from B symptoms at some time. This corresponds with the finding that seven of these patients had presented with documented opportunistic infections. On the other hand all patients without B symptoms (classified as A) were still alive at the end of the observation period. Thus, the median survival of 12 patients without systemic symptoms was 100% at 32 months, whereas out of 25 patients with B-symptoms 14 (56%) died within the same time period.

TABLE 1. Clinical findings in 37 patients with Kaposi's sarcoma and AIDS

	No. of patients	%
Opportunistic infections		
Pneumocystis carnii pneumonia	12	32
Ulcerating herpes simplex	7	19
Candida oesophagitis	3	8
Atypical mycobacteriosis, disseminated	1	3
Toxoplasmosis*	1	3
Cytomegalovirus infection, disseminated*	1	3
Other infections with skin manifestions		
Herpes zoster infection	3	8
Herpes simplex (non ulcerating)	12	32
Verruca vulgaris	6	16
Condyloma acuminata	2	5
Molluscum contagiosum	5	14
Candidosis, oral, genital or intestinal	24	65

* Found at autopsy in a patient who developed *Pneumocystis carnii* pneumonia.

Staging of patients according to the Laubenstein classification,[10] is listed in Table 2. From the first examinations at the beginning of the study over a period of a maximum of 32 months, 25 patients remained stable, while 12 patients deteriorated. Changes from one stage to another occurred only for the worse; improvement in stages was never observed. Almost all patients with opportunistic infections suffered from B symptoms.

There were no significant differences in the T4/T8 ratios or absolute T4 counts between patients in the A stage of each grade and those in the B stage (Table 3). This is probably because

TABLE 2. Classification of Kaposi's sarcoma in 37 patients with AIDS according to the Laubenstein classification[10]

		No. of patients at end of observation period (1–32 months)								
		Stage								
Disease stage	No. of patients at beginning of observation period	IA	IB	IIA	IIB	IIIA	IIIB	IVA	IVB	With opportunistic infection
IA	3	2	1	...	...	...	...	...	...	...
IB	0	...	...	...	...	...	...	...	...	...
IIA	9	...	...	6	2	...	1	...	...	4
IIB	7	...	...	...	6	...	1	...	...	4
IIIA	8	...	...	...	...	3	4	...	1	3
IIIB	6	...	...	...	...	...	4	...	2	5
IVA	2	...	...	...	...	...	...	2	...	...
IVB	2	...	...	...	...	...	...	...	2	1
Total	37	2	1	6	8	3	10	2	5	17

TABLE 3. T4/T8 ratio and absolute T4 lymphocyte counts in different clinical stages of Kaposi's sarcoma in AIDS

	Disease stage							
	IA	IB	IIA	IIB	IIIA	IIIB	IVA	IVB
T4/T8 ratio*	0·53 ($n=3$)	0·30 ($n=1$)	0·57 ($n=8$)	0·38 ($n=7$)	0·46 ($n=7$)	0·19 ($n=3$)	0·57 ($n=2$)	0·30 ($n=1$)
absolute T4 counts (cells/μl)*	364 ($n=3$)	105 ($n=1$)	330 ($n=5$)	136 ($n=5$)	213 ($n=6$)	139 ($n=3$)	288 ($n=2$)	157 ($n=1$)

* Overall mean for each disease stage calculated from individual patient means.

of the small numbers of patients, because lower T4/T8 ratios and absolute T4 counts were found in B stage patients than in A stage patients at each grade (Figs 1 and 2). Comparison, however, of the mean values for all patients in stage A with all those in stage B showed significantly higher absolute T4 counts in the patients without systemic symptoms (stage A) ($P=0·02$). The difference in the T4/T8 ratio was not significant ($P=0·2$).

Delayed type hypersensitivity was examined in 27 patients using an intradermal test with recall antigens ('Multitest Merieux'). At the beginning of the observation period 16 patients (59%) were anergic, five (19%) showed diminished cutaneous responses and six (22%) showed normal responses. The cutaneous responses are listed according to disease stage in Table 4, which also indicates the numbers of patients whose category of skin reactivity changed during observation period. Again, only progression towards anergy was found; no changes occurred in the other direction. There was no apparent relationship of the degree of cutaneous response with any of the disease grades nor with A or B stages.

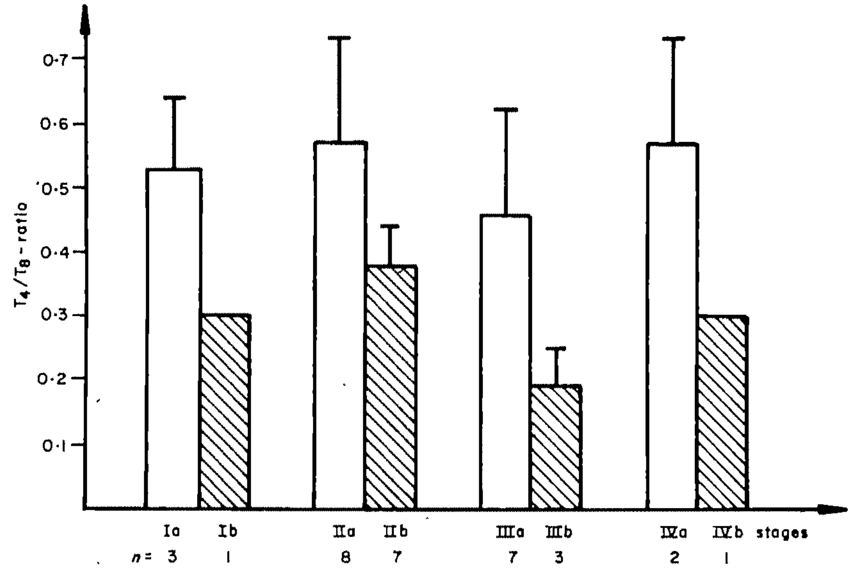

FIGURE 1. T4/T8 ratios at different clinical stages of Kaposi's sarcoma in AIDS. Bars indicate means ± SEM.

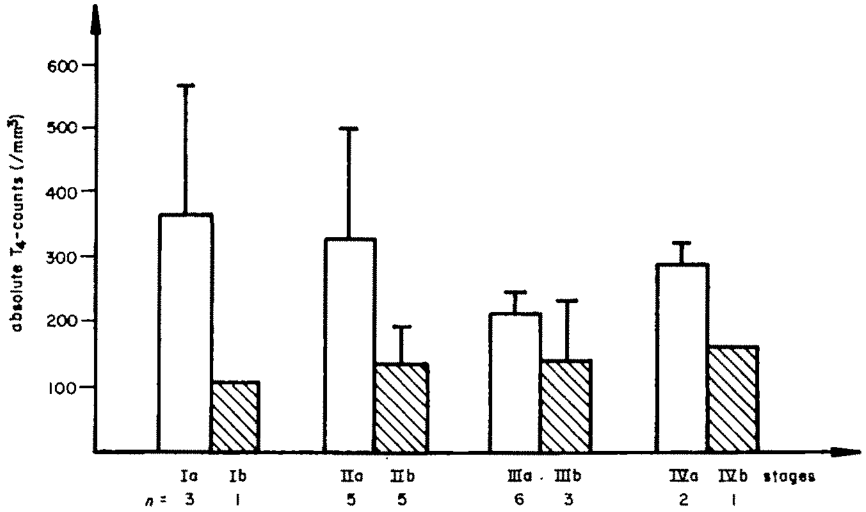

FIGURE 2. Absolute T4 lymphocyte counts in different clinical stages of Kaposi's sarcoma in AIDS. Bars indicate means ± SEM.

TABLE 4. Delayed type cutaneous reactions at different clinical stages of Kaposi's sarcoma in AIDS

Skin reactivity*	No. of patients in clinical stages IA	IB	IIA	IIB	IIIA	IIIB	IVA	IVB
Normergy	1	···	4(2)	···	···	1(1)	···	···
			↓			↓		
Hypoergy	···	···	···	2(1)	1	1	···	1
				↓				
Anergy	2	···	4	5	3	1	1	···
Total	3	0	8	7	4	3	1	1

* Sum of positive skin reactions: > 10 mm = normergy; 2–9 mm = hypoergy and 0–1 mm = anergy. Arrows indicate numbers of patients who changed category during the period of the study.

When skin reactivity was plotted against T4/T8 ratios measured at the same time, there was no significant correlation (Fig. 3). However, there was a trend towards a positive correlation between delayed type hypersensitivity and absolute T4 counts ($r = 0·59$; Fig. 4).

DISCUSSION

The results of this study demonstrate clear differences in the cellular immune parameters T4 numbers, T4/T8 ratio and intradermal delayed type hypersensitivity, between subgroups of patients with Kaposi's sarcoma and AIDS. There was no apparent relationship between these

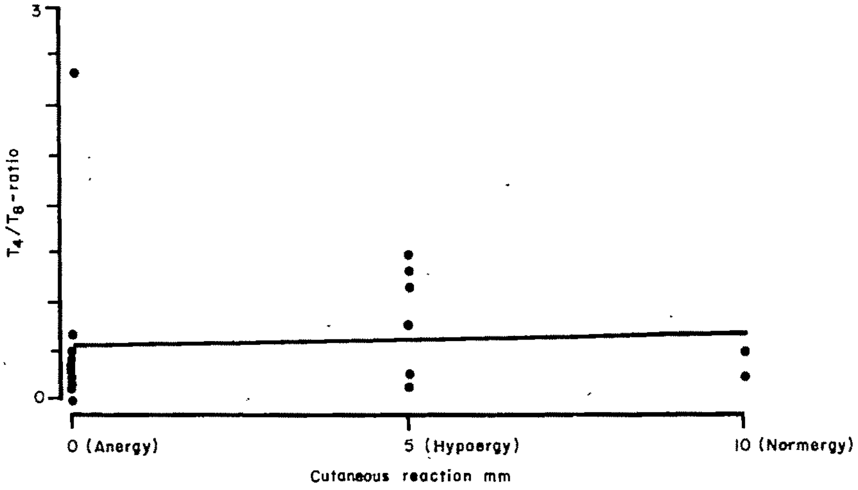

FIGURE 3. Relationship between delayed type cutaneous reactions (sum of diameters of positive reactions in mm) and T4/T8 ratio in patients with Kaposi's sarcoma in AIDS. ($r = 0{\cdot}084$).

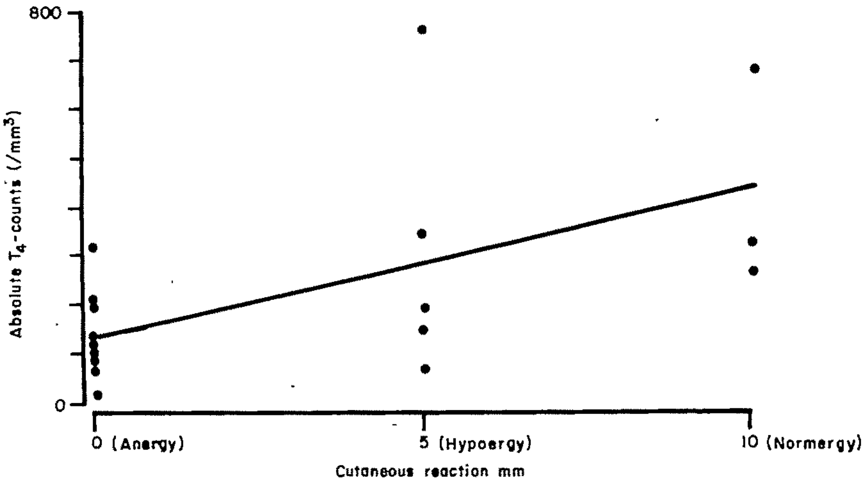

FIGURE 4. Relationship between delayed type cutaneous reactions (sum of diameters of positive reactions in mm) and absolute T4 counts in patients with Kaposi's sarcoma in AIDS. ($r = 0{\cdot}585$).

immune parameters and the extent of Kaposi's sarcoma lesions as graded by the Laubenstein classification. Patients suffering from B symptoms, however, had lower T4 values than patients in the A subgroup, irrespective of the extent of the lesions (including visceral involvement). Thus, widespread Kaposi's sarcoma in AIDS can occur with almost normal cellular immune parameters.

Kaposi's sarcoma develops in about 30% of all AIDS patients and affects almost exclusively homosexual men as was the case in our patients. It has been observed that AIDS patients with Kaposi's sarcoma alone have a better prognosis and a longer life expectancy than those who have opportunistic infections also.[9,12] Krigel[13] reported an 80% survival at 28 months in patients with epidemic Kaposi's sarcoma (EKS) compared with a survival rate of less than 20% in patients with EKS and concomitant opportunistic infections. Another early study showed similar results.[12] The classification of Kaposi's sarcoma by Krigel, Laubenstein and Muggia[10] includes A (no systemic symptoms) and B symptoms (presence of systemic signs). In the present study 25 patients (65%) had developed B symptoms within 32 months, of whom 17 presented with opportunistic infections. Of the 25 patients with B symptoms, 14 died within 32 months at least seven of these having suffered opportunistic infections. Thus, the survival rate of patients with opportunistic infections. Of the 25 patients with B symptoms, 14 died within 32 months, at least seven of these having suffered opportunistic infections. Thus, the survival rate of patients an earlier study: of 15 patients with EKS, those without systemic signs had a survival rate of 100% whereas those with B symptoms had only 50% survival at 28 months.[14] These results suggest that the onset of systemic symptoms represents an important prognostic factor in the course of EKS in AIDS.

However, the extent of the skin lesions, including gastro-intestinal involvement, did not have any prognostic value. Gastrointestinal tract involvement has been described in about 45% of cases of EKS[10,15] and was not related to the survival rate.[15]

These clinical findings are in agreement with the immunological findings; patients with EKS alone seem to be less immunosuppressed than those presenting with opportunistic infections also.[16] Among the many immunological abnormalities described in HIV infection[9,11] the T4/T8 ratio, absolute T4 numbers and intradermal reactivity are most widely used. The T helper/T suppressor (T4/T8) ratio and the absolute T4 numbers are comparatively higher in patients with EKS as the only manifestation of AIDS.[9,17] The lowered T4/T8 ratio could also be due to a relative increase in the numbers of T8 suppressor/cytotoxic lymphocytes which is seen in other viral infections but also in early stages of HIV infection.[11,17-19] Therefore, the absolute T4 lymphocyte count seems to be a more reliable measure of immunosuppression in AIDS.[17,19]

In the present study patients with systemic symptoms (B stages) showed a marked reduction of the T4/T8 ratio and absolute T4 numbers compared with the patients with no systemic signs (A stages). When results for patients in all clinical stages were analysed together there was a significant difference between A and B patients with respect to absolute T4 numbers, but not the T4/T8 ratio. Thus the decrease in T4 lymphocyte numbers shows closer relationship with onset of B symptoms in EKS than does the decrease in the T4/T8 ratio.

Additionally, delayed type hypersensitivity, as tested with recall antigens, was found to be depressed already at the first examination in many patients (59%). This corresponds with previous findings in patients with EKS[2] and with lymphadenopathy-syndrome.[20] Thus, cutaneous anergy seems to be an early indicator of immunological dysfunction. However, cutaneous responses can also be intact in the early stages of the syndrome.[21]

Finally, concerning the clinical classification of Kaposi's sarcoma, about one third (12 of 37) of the patients studied showed progression of the disease and 38% died during the period of observation. Other authors give a higher percentage of progression over similar time periods. Brodt *et al.*[22] also observed patients with opportunistic infections. These authors stated that the onset of all the manifestations of HIV infection depend on the condition of the patient at his first examination, the degree of immunosuppression and on the period of observation.

In the present study there was no correlation between delayed type hypersensitivity and the T4/T8 ratio, but there was a tendency towards a relationship with T4 numbers.

Thus, a defect in cellular immunity appears to be a prerequisite for Kaposi's sarcoma. However, progression of the lesions seems to be independent of the degree of immunodeficiency, at least with respect to T cell subpopulations and skin reactivity. However, more exhaustive study of T lymphocyte subpoplations, for example, expansion by two colour fluorescence,[23] might reveal a relationship with disease stage.

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A new development in UVB phototherapy of psoriasis

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SUMMARY

We have compared the therapeutic effectiveness of a new UVB fluorescent sunlamp, the Philips TL-01 lamp, which emits a narrow peak around 311–312 nm, with the currently used Philips TL-12 lamp, in 10 patients with psoriasis. We also compared the tumour inducing capacity of the two lamps in hairless mice.

The therapeutic effect of the TL-01 lamp was superior to that of the TL-12 lamp in nine of the 10 patients. In the mice, the median tumour induction time was significantly longer in animals exposed to the TL-01 lamp.

Phototherapy with the new type of lamp requires a higher dose than phototherapy with the usual broadband UVB sources. In practice this means that more lamps are needed in the light cabinet. However, the new lamps appear to provide more effective and safer phototherapy for psoriasis.

Several investigators have reported the influence of wavelength on therapeutic efficacy in phototherapy, mainly with regard to psoriasis. Fisher[1] found 313 nm more effective than longer wavelengths in the UVA region. Parrish and Jaenicke[2] determined an action spectrum for phototherapy of psoriasis. The most effective wavelengths were between 295 and 313 nm; the therapeutic effectiveness in that range resembled the erythemal action spectrum, peaking near 300 nm. In the four patients tested, the ratio of lowest effective daily dose (LEDD) to the minimal erythema dose (MED) was smallest for 313 nm, and significantly < 1 at this wavelength only ($P < 0{\cdot}05$). A ratio < 1 indicates that suberythemic doses lead to clearing. These results give the impression that the wavelength region around 313 nm is relatively effective in the treatment of psoriasis. Amlong *et al.*[3] found by simple calculations based on the same data,[2] that the wavelength range 304–314 nm may be best for psoriasis phototherapy; the wavelength region around 310 nm would give the optimal ratio of therapeutic and erythemal effectiveness. Van Weelden *et al.*[4] compared combinations of UVA, UVB and UVC in phototherapy of psoriasis. The UVB region turned out to be the most effective using the full spectrum of Westinghouse FS40T12 fluorescent sunlamps. Addition of UVA to the UVB did not improve the therapeutic

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effectiveness. The addition of UVC, however, decreased the effectiveness; the shorter wavelengths contributed so much to the erythematogenic effectiveness that this limited the exposures to the longer wavelengths. This also indicated that UVC itself contributed little or nothing to the therapeutic effectiveness.

These results suggested that an improvement in the effectiveness of the UVB lamps might be achieved by filtering out the shorter, most erythematogenic wavelengths (Fig. 1).

The lamp developers at Philips came up with an interesting solution. Rather than creating a tube with a different filtering glass they experimented with phosphors and the result was a fluorescent lamp particularly suitable for the present study. The spectrum of this lamp, the Philips TL-01, is shown in Figure 2; the lamp is expected to come into production by the end of 1988.

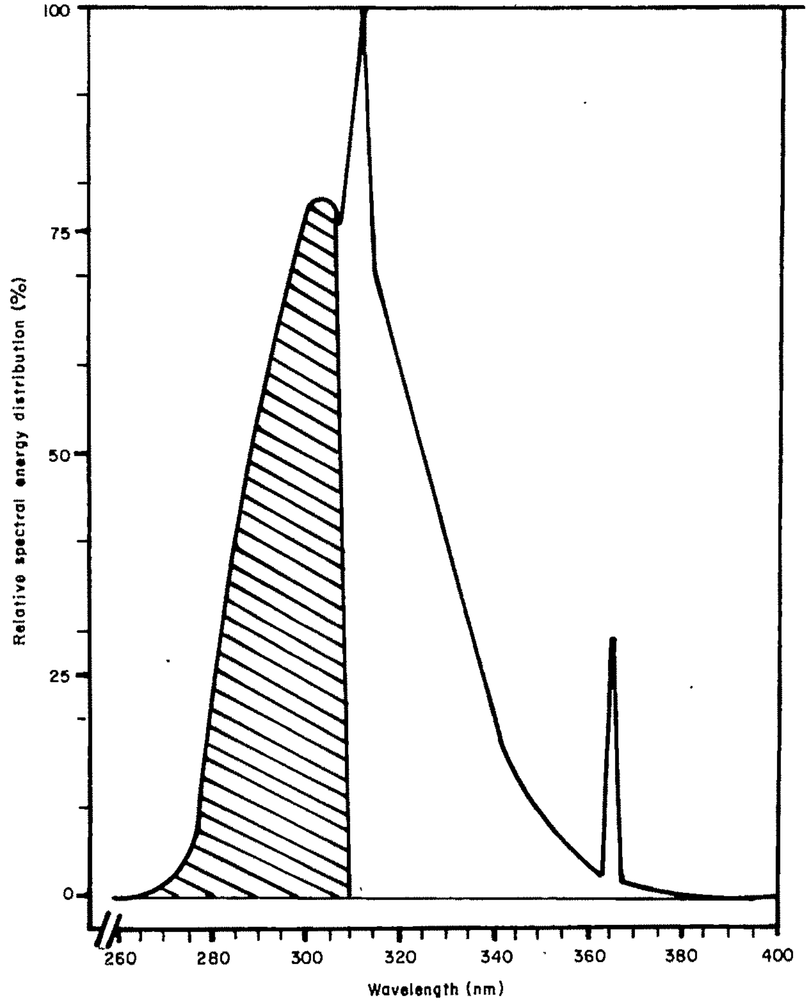

FIGURE 1. Relative spectral energy distribution of a Philips TL-12 40W UVB sunlamp. Filtering out of the shaded part of the spectrum might be an improvement for use in phototherapy of psoriasis.

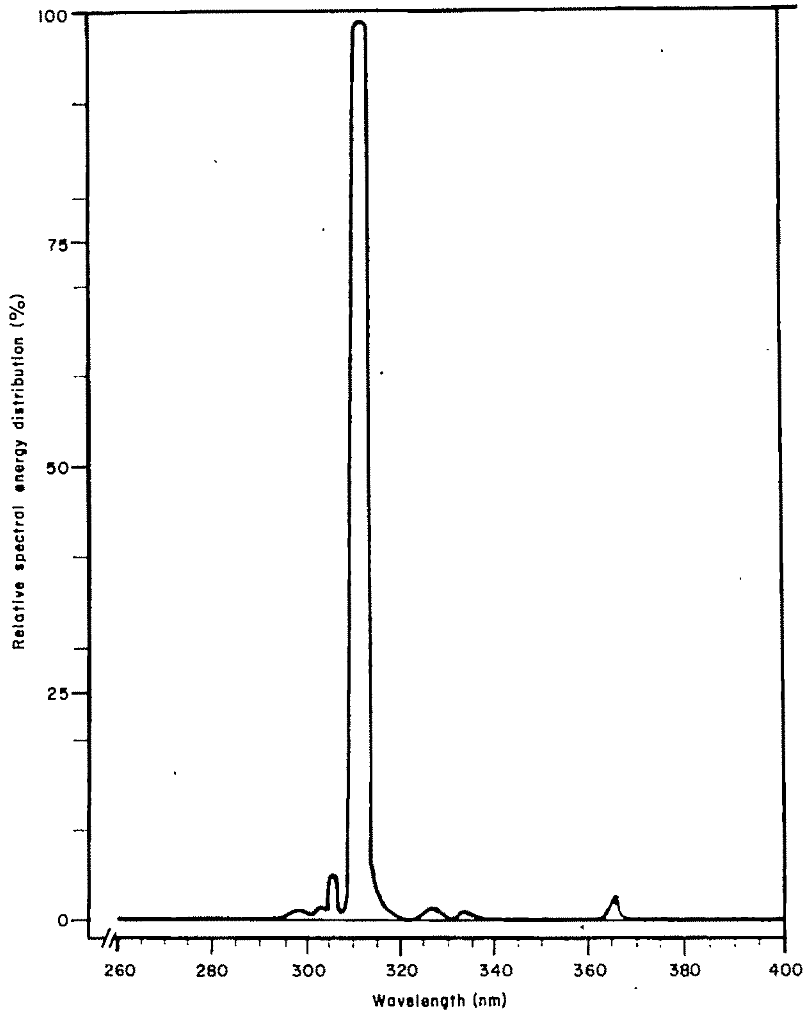

FIGURE 2. Relative spectral energy distribution of the experimental fluorescent lamp, Philips TL-01/40W.

In the present study we compared the effectiveness of the new lamp with the Philips TL-12 fluorescent sunlamp, in patients with psoriasis. We also compared the tumour promoting activity of the two lamps in mice.

METHODS

Ten patients, six male and four female, (age range 13–77 years) with widespread symmetrical psoriasis vulgaris took part in the study. Disease duration ranged from 1 to 61 years, (mean 17·5 years). The patients were hospitalized throughout the investigation.

The patients were exposed to UV radiation from fluorescent light tubes in a square light cabinet with inner dimensions 1·30 × 1·30 m, with reflecting walls packed with lamps of two types, Philips TL-01 and TL-12. The spectral composition of the UV radiation in the light cabinet was selected by switching on the lamps of the desired type.

In all patients, 1% salicylic acid in petrolatum was applied to the whole body daily, to remove the psoriatic scales and to prevent dryness of the skin, which is a common effect of regular exposures to UV radiation. The salicylic acid was applied after UV exposures, so that it was unlikely to influence the effect of irradiation.

Light sources

The spectrum of the new lamps used in the study is shown in Figure 2.

The spectrum is dominated by a strong and narrow peak (bandwidth 2·5 nm) around 311–312 nm, with a second peak around 305 nm. Compared with the usual fluorescent UVB lamps, such as the Philips TL-12, the TL-01 has much less output at wavelengths of 300 nm and below and more at 311–312 nm.

The relative spectral distribution measurements were performed with a monochromator (Jarell-Ash, model 84-425 SP), bandwidth 1·2 nm, in combination with a photomultiplier (Hamamatsu R212).

The irradiance in the light cabinet was measured for the two different light sources, with all lamps of the particular type switched on. In both cases the output of the lamps over the full spectral range was measured with a Kipp thermopile E11. The irradiances in the light cabinet were checked routinely with a Waldmann AG UVB detector (Schwenningen, FRG). In the animal experiments, as is customary,[5,6] the constancy of the UVB irradiance was checked regularly by using the UVB detector head of a Robertson-Berger meter (Solar Light Company) in combination with a Kipp Micrograph micro-ammeter.

Therapeutic effectiveness

A paired comparison of therapy with the two types of lamp was performed.[7,8] The patients received treatment with the TL-01 lamps on the one half of the body and with the TL-12 lamps on the other half. The half not being irradiated during each treatment was shielded by an overall cut in half lengthwise. The treatments were randomized to the two halves of the body.

The UVB phototherapy was given to elicit slight erythema after each exposure.[4] Before the first exposure, the minimal erythema dose (MED) was determined. The first therapeutic exposure was 70% of the MED. The successive doses were given on the basis that, if the previous exposure had induced no perceptible effect, the exposure time was increased by 40%, if the previous exposure time had caused a slight erythema, the exposure time was increased by 20% and in cases of marked erythema the same exposure time was used again.

The two therapies were given three times a week in eight patients; in two patients five exposures were given per week. In these two patients, in order to produce only slight erythema, the increments in exposure time were smaller. During a course of treatments, there was a tendency for the increments to become smaller, finally, reaching a constant level of tolerance induced by regular exposure.

The therapeutic effects were assessed regularly by at least two experienced clinical observers, who had no knowledge of which therapy was being administered to which side in each patient. In a previous study[4] we found that cross-over effects did not play an important role in investigations of this type. On the basis of assessments of erythema, scaling, infiltration and extent of the lesions, the observers recorded which side was better, or that there was no difference.

Carcinogenic effects

Animal experiments, to compare the carcinogenic effects of the TL-01 and TL-12 lamps, were performed on albino hairless mice (strain Skh-hr 1). Male and female mice were used. One group of 24 mice was exposed to the TL-12 lamps and a second group of 24 mice was exposed to the TL-01 lamps.

The mice were kept separately in cages that were subdivided into 12 compartments; the animals had free access to drinking water and mouse chow. The mice were subjected 7 days a week to dorsal exposure to UV-radiation from the light sources situated above the cages.[9] Two TL-12 lamps or six TL-01 lamps were used. The irradiance was regulated by means of a Triac light dimmer.

In a preliminary study of acute effects, mice of the same strain received a series of test irradiation doses on their backs. Because of the relatively small skin area available 4 fields of 5 × 5 mm were exposed to doses increasing by a factor of 2. The remainder of the skin was shielded by opaque adhesive tape. During these exposures the animals could move around freely in their cage compartments, as during the chronic exposures. All observations were made by at least four experienced observers who had no knowledge of the type of irradiation or the dose. The doses necessary to induce just perceptible reactions, erythema and oedema (MEdD), were determined in the preliminary experiments. The daily doses given in the carcinogenesis experiments were $0{\cdot}56 \times$ MEdD, with each light source.

All skin reactions were regularly assessed during the chronic experiments. When most of the animals had developed tumours larger than 4 mm the experiments were stopped.

Histopathology of the tumours

At the end of the experiment the animals were sacrificed and samples of the larger tumours were excised and sectioned for histopathological examination, after staining with haematoxylin and eosin.

RESULTS

Patients

Treatment of each patient was continued until the comparison of the two sides gave the same differences twice in a row. 'No difference' was recorded when there was no difference between the two sides after 10 treatments (Table 1). The assessments of the clinical observers were always in good agreement. After completion of the comparisons in each patient, treatment with the lamp which gave the best therapeutic result was applied to the whole body.

TABLE 1. Comparison of therapeutic effectiveness of TL-01 and TL-12 lamps in 10 patients with psoriasis

	No. of patients
TL-12 better	0
No difference	1
TL-01 better	9

The TL-01 lamps were found to be more effective than the TL-12 lamps ($P < 0{\cdot}05$; sign test). The differences were not very large, but the TL-01 lamp gave a better result in nine of the 10 patients.

The mean minimal erythema dose (MED) (± SD) before therapy was 76 ± 12 mJ/cm^2 for the TL-12 lamps and 410 ± 25 mJ/cm^2 for the TL-01 lamps. The ratio of the MEDs of the two lamps was $5{\cdot}4 \pm 0{\cdot}62$, if the full spectral ranges of both lamps were measured. When the irradiances were measured with a Waldmann UVB detector this ratio was $7{\cdot}0 \pm 0{\cdot}8$. The MED in psoriasis patients tends to be somewhat higher than in healthy subjects. In a group of healthy young volunteers we have found the mean MED with the TL-12 lamp to be 55 mJ/cm^2.

After the patients had cleared or improved to a satisfactory level, the treatment, in all cases with the TL-01 lamps, was discontinued. The mean time before a recurrence of the psoriasis, i.e. before the patients needed treatment again, was 5·5 months; range 5–10 months.

Animal study

The experiments on mice showed that, with both types of lamp, the acute skin reactions were erythema and oedema. The ratio of the doses required with the two types of lamp for acute effects in mouse skin was $5{\cdot}8 \pm 1{\cdot}4$ in the case of full spectral range measurements, which was similar to the ratio of the MEDs in human skin. On this basis, the daily doses for the carcinogenesis experiments, 0·56 MEdD, were 355 mJ/cm^2 for the TL-01 lamps and 61·5 mJ/cm^2 for the TL-12 lamps.

All the animals developed tumours. The median time for induction of the smallest visible tumours was significantly longer in the animals exposed to the TL-01 lamps than in the animals exposed to the TL-12 lamps (Table 2). For larger tumours the median induction time with the TL-01 lamps was longer than that with the TL-12 lamps, but not significantly so ($P > 0{\cdot}05$).

The prevalence of tumour-bearing mice, expressed as a percentage of number of mice in the group, can be presented as a function of time by a cumulative log-normal distribution[5,10] (Fig. 3). Using a probability scale for prevalence and a log scale for time, a linear relationship can be obtained.[10] The tumours in the mice receiving TL-12 irradiation appeared significantly earlier than the tumours in the mice exposed to TL-01 irradiation ($P < 0{\cdot}01$). However, by the time all the mice in the group exposed to the TL-12 lamps had developed at least one tumour almost all the mice exposed to the TL-01 lamps had done so as well (Fig. 3).

TABLE 2. Median tumour induction times in hairless mice irradiated with TL-12 or TL-01 lamps

	TL-12		TL-01		
Tumour size	t_m (days)*	$\ln t_m \pm$ SD**	t_m (days)*	$\ln t_m \pm$ SD**	P†
<1 mm	104	$4{\cdot}64 \pm 0{\cdot}21$	117	$4{\cdot}76 \pm 0{\cdot}14$	$<0{\cdot}05$
≥ 1 mm	114	$4{\cdot}74 \pm 0{\cdot}19$	125	$4{\cdot}83 \pm 0{\cdot}11$	NS
≥ 2 mm	134	$4{\cdot}90 \pm 0{\cdot}18$	145	$4{\cdot}98 \pm 0{\cdot}13$	NS

*t_m = median induction time, i.e. the time to appearance of tumours in 50% of the group of mice.

** SD = standard deviation of the log-normal distribution.

† P = Student's t-test.

NS = not significant.

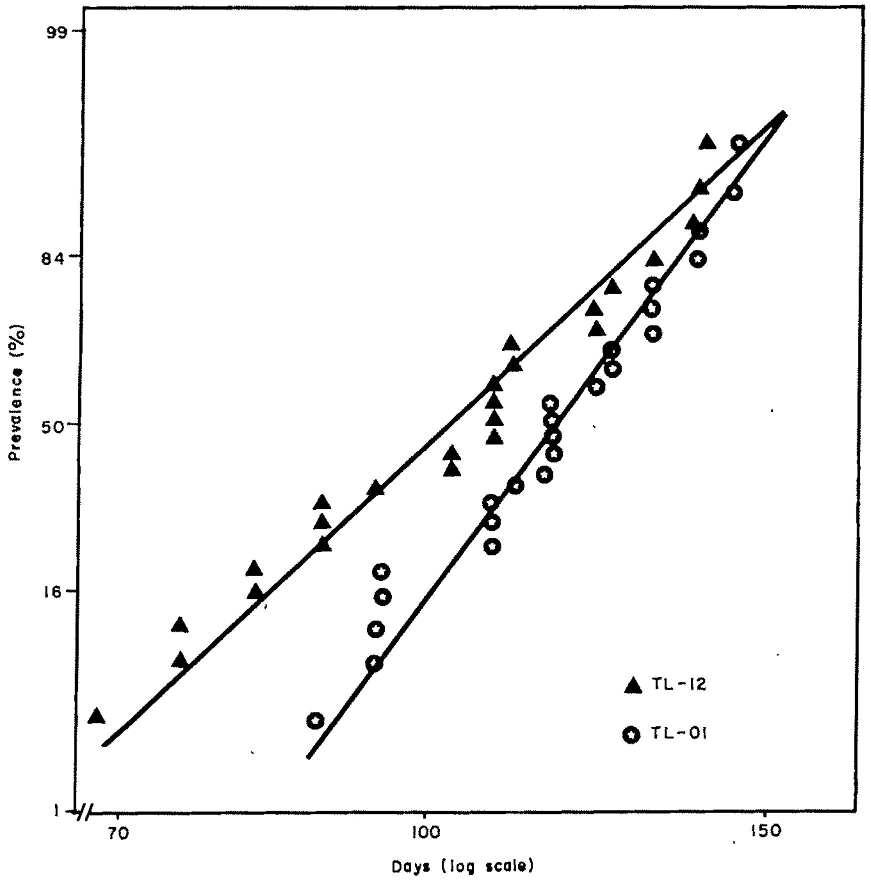

FIGURE 3. Prevalence of tumour-bearing mice, on a probability scale, against time in days on a log-scale, for mice exposed to TL-12 lamps (▲) and TL-01 lamps (○).

In a related study Sterenborg *et al.*[11] performed additional experiments on photocarcinogenesis with the TL-01 lamps. These results and those from the present study showed that the dose-response relationship was similar to that found with broad-band UVB from Westinghouse FS40 sunlamps.

Histopathology

Clinically, verrucous tumours were observed as well as keratoacanthoma- and cornu cutaneum-like tumours. Samples for histopathology were taken only from tumours larger than 4 mm diameter, because it is known that the diagnosis can be established with more certainty from larger tumours. Almost all the tumours examined turned out to be squamous cell carcinomas, some of them invading the dermis. In only 10% of the tumours examined could the diagnosis squamous cell carcinoma not be stated with certainty. There was no difference in this respect between the tumours induced by the TL-01 and by the TL-12 lamps.

DISCUSSION

In the present study, phototherapy with TL-01 lamps was more effective than treatment with TL-12 lamps. These results are supported by those in another series of patients,[12,13] where sequential treatments of the whole body with the two types of lamps were compared. In that study, in seven out of eight patients the number of exposures required to produce 80% improvement was significantly smaller with the TL-01 lamps than with the TL-12 lamps.

The mean remission period (± SD) after treatment with the TL-01 lamps (5·5 ± 3·5 months) was not significantly different from the remission periods observed with PUVA (5·2 ± 2·8 months) and with broad-band UVB from currently used sunlamps (5·4 ± 3·4 months) in a previous study.[4] Therefore, the remission period is not an important consideration in the choice of therapy.

In the phototherapy of psoriasis, the erythema reaction is important as well as the therapeutic effect. Exposure to several MEDs leads to discomfort, and in some cases even to the induction of new lesions, (the Koebner phenomenon). Doses much smaller than 1 MED are not therapeutically effective.[1,2] Hence, the clinical practice of administering doses of UV resulting in a slight erythema. This practice allows direct checking that the doses given are in the range intended. At the same time it provides a guideline for the dose increments required during a course of treatment, to allow for adaptation of the skin to the UV radiation.

This guideline was followed consistently in the present study, allowing comparison of the effectiveness of the different phototherapies. Otherwise, the result of a therapy could be governed more by the dose-regimen, than by other features. The results have shown that, if compared in terms of MED's, the TL-01 lamps appear to be more effective than the TL-12 lamps. On the other hand a larger dose is needed; the wavelengths 311–312 nm are less effective than the shorter wavelengths in terms of the dose required to induce just perceptible erythema, and also to achieve a therapeutic effect. In practice this means that about 6 times as many lamps are needed for phototherapy with TL-01 lamps as with the TL-12 lamps, with the same exposure times. This would make UVB phototherapy with TL-01 lamps more expensive, but not excessively so.

The most important evaluation is not effectiveness versus costs, but effectiveness versus risks. The present study suggests that the TL-01 lamps are slightly less carcinogenic than the TL-12 lamps for erythematogenically equivalent daily doses. This conclusion should be drawn with caution, however, because the difference was statistically significant only for the smallest tumours. Also, although the tumours in the mice exposed to the TL-12 lamps appeared before the tumours in the mice exposed to the TL-01 lamps, by the time all the mice in the group exposed to the TL-12 lamps had at least one tumour they had been almost completely caught up by the group exposed to the TL-01 lamps.

Thus, the TL-01 lamp appears to be not only therapeutically more effective, but also safer and in our opinion, a promising lamp for phototherapy of psoriasis and possibly other photobiological applications. The manufacturers still consider the TL-01 lamp as experimental; they have however, produced a new series of lamps for testing in a multicentre trial. By the end of 1988 the lamp is expected to become commercially available.

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Cancer of the skin, particularly basal and squamous cell carcinoma, occurs primarily in people with fair skin[1] who burn easily following exposure to ultraviolet radiation (UVR). In contrast, the incidence of skin cancer in inherently dark-skinned people is low.[2] Melanin is synthesized by melanocytes in response to UVR[3] and is thought to protect epidermal cells against damage to their genetic material by absorbing UVR and thereby reducing its penetration into the skin.[4,5] Thus darkly pigmented skin is more resistant to the effects of UVR.[6,7] In a statistical study on the effects of UVR on epidermal cells,[8] we found that HLA-DR+ (DR+) and T6+ Langerhans cells (LC) were depleted to a significantly greater extent in subjects of Celtic descent with fair skin than in subjects of Asian or Aboriginal descent with dark skin. This suggested that constitutive dark pigmentation provided some protection against the effects of UVR on epidermal DR+ and T6+ cells. In the epidermis, only Langerhans cells and so called 'indeterminate cells' (IC), thought to be LC precursors,[9] express T6 surface antigens.[10] These cells form the outermost part of the human immune system,[11] and are essential for the recognition of cutaneously introduced antigens and their presentation to T lymphocytes.[12,13] In the case of absence, reduction or dysfunction of immunocompetent (i.e. DR+) LC, such as that induced by UVR exposure,[12,13] specific tolerance to antigens can be induced,[12,14] a situation ideal for the promotion of unhindered growth of abnormal epidermal cells.

The aims of the present study were to examine and compare the responses of epidermal LC and melanocytes to relatively small doses of UVR (combined UVA and UVB), in subjects of widely differing skin pigmentation, and to substantiate our previous finding[8] that neither inherently dark skin pigmentation nor UVR-induced increases in pigmentation are sufficient to prevent the depletion of immunocompetent LC. To do this, the ultrastructure of LC and melanocytes was examined in dark-skinned subjects of Aboriginal descent and fair-skinned subjects of Celtic descent before and after UVR.

METHODS

Subjects

Seven Australian volunteers, four of Aboriginal and three of Celtic descent participated in the study. The Aboriginal subjects (three women and one man) had heavily pigmented skin and ranged in age from 28 to 55 years. The fair-skinned Celtic subjects (all men) ranged in age from 36 to 65 years.

Design of study

The study was carried out in Sydney (Australia) from April to July. Observations were made on epidermis from previously unexposed buttock skin. This skin was exposed to UVR for 7 min on each of 3 consecutive days. Subjects took care not to expose themselves to sunlight during the experiment. Skin biopsies were taken prior to UV exposure, and 2 h and 5 days after the third exposure.

UV irradiation

The UV source was designed to simulate natural ultraviolet radiation and consisted of six Sylvania sunlamps emitting UVA (320–380 nm) 1·386 mW/cm^2 (total dose 1·75 J/cm^2) and UVB (280–320 nm) 0·256 mW/cm^2 (total dose 322 mJ/cm^2) at the level of the exposed buttock. The sunlamps were mounted on a frame 1 m above an examination table on which the volunteers were exposed.

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Ultrastructural changes in epidermal Langerhans cells and melanocytes in response to ultraviolet irradiation, in Australians of Aboriginal and Celtic descent

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SUMMARY

The effects of exposure to small doses of artificial ultraviolet radiation (UVR) on the ultrastructure of epidermal Langerhans cells (LC) and melanocytes were studied in two groups of Australian subjects, one of Aboriginal and the other of Celtic descent.

UV exposure induced an apparent depletion of LC in the epidermis of both groups. However, LC depletion in the Aboriginal subjects was associated with apoptosis, whereas organelle and membrane disruption in the LC of Celtic subjects suggested a reduction by direct cellular damage. LC in Aboriginal epidermis tended to become relocated at more superficial levels following UV exposure, and their Birbeck granules became more numerous. LC in Celtic epidermis appeared to become relocated in a basal location and contained fewer Birbeck granules. The central lamina of the Birbeck granules in Aboriginal LC, which was more electron-dense than that in Celtic subjects prior to UV treatment, was temporarily lost following treatment, while the ultrastructure of Birbeck granules in Celtic LC was unchanged. LC and 'indeterminate cells' in intimate association with lympocyte-like cells occurred in the basal layer of Celtic epidermis 5 days after exposure. These complexes were not observed in Aboriginal epidermis although isolated lymphocyte-like cells were observed in the same location.

Melanocytes in Aboriginal epidermis contained greater numbers of melanosomes than those in Celtic epidermis throughout the experiment. Inactive epidermal melanocytes in Celtic subjects initially responded to UV exposure with a slight increase in melanosome content followed by a substantial further increase, whereas active melanocytes in the Aboriginal subjects showed the opposite response.

The implications of the different responses of LC and melanocytes in the two groups, in relation to immunological function of the epidermis and the marked racial difference in the incidence of skin cancer, are discussed.

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Electron microscopy

Skin shave biopsies approximately 0·5 cm^2 were taken from the buttock with a scalpel blade after subcutaneous injection of 1% lignocaine. The specimens were cut into 1 mm^3 pieces and processed for electron microscopy using a two-stage glutaraldehyde/paraformaldehyde fixation, post-fixation in osmium tetroxide, dehydration in ethanol and propylene oxide and impregnation and embedding in Araldite as described previously.[15] Thin sections (60–80 nm) cut from two pieces of each skin sample, were stained with uranyl acetate and lead citrate and examined with a Jeol 100S electron microscope. The numbers of LC in measured lengths of epidermis were counted during direct observation with the electron microscope. Only those LC sectioned through the nucleus were included. The number of Birbeck granules in LC was also assessed quantitatively by counting the number present in each LC sectioned through the nucleus. Difficulty was encountered in recognizing granules sectioned obliquely or tangentially; only clearly identifiable granules were counted.

RESULTS

Aboriginal subjects

Prior to UV exposure. LC were located at fairly regular intervals in the epidermis with a mean (± SEM) of 3·6 ± 0·4 cells/mm length of epidermis. Most of them (75%) were situated one or two cells above the basal lamina (Fig. 1) and they contained large lobulated nuclei surrounded by organelle-rich cytoplasm which extended into long horizontally orientated dendrites. Birbeck granules were sparse in these cells (mean 2·7 ± 0·4/LC) and their central laminae were more electron-dense than that usually encountered in Caucasian LC (Fig. 1). Melanocytes containing moderate numbers of melanosomes were located in the basal layer of the epidermis. Melanosomes were also distributed throughout the epidermis, mostly within the keratinocytes of the basal layer. Electron-dense material resembling compacted melanosomes occupied some intercellular spaces (Fig. 1) and a few melanosomes had been phagocytosed by LC. Lymphocyte-like cells were occasionally observed in the basal layer, protruding into the dermis (Fig. 2). No evidence of apoptotic cell deletion was observed.

Two hours after UV exposure. None of the Aboriginal subjects developed visible erythema. LC were less numerous (mean 1·4 ± 0·5/mm) and most of them (81%) were now located in mid epidermis. Their structure was, in general, similar to that encountered prior to UV exposure. However, Birbeck granules were more numerous (mean 22·7 ± 1·2/LC) and their central laminae, which in unexposed cells were very electron-dense, were no longer visible (Fig. 3). The melanosome content of melanocytes and keratinocytes had increased markedly and occasional lymphocyte-like cells were again encountered in the basal layer. There were no obvious signs of cellular damage. However, one LC in an early phase of apoptotic regression was observed at a suprabasal level of the epidermis in one subject and two apoptotic bodies of probable LC origin which had been phagocytozed by a basal keratinocyte were observed in another subject.

Five days after UV exposure. The LC numbers were similar to those found prior to exposure (mean 3·6 ± 0·6/mm), but their location varied considerably from mid epidermis (54%), to the basal layer (14%). Some dendrites extended almost to the cornified layer. The number of Birbeck granules in LC also varied (mean 19·1 ± 1·8/LC) and their central laminae were visible, albeit of very low electron density. Another LC in an early phase of apoptosis (Fig. 4) was observed in the same location as that 2 h after UV exposure. A further, but less substantial

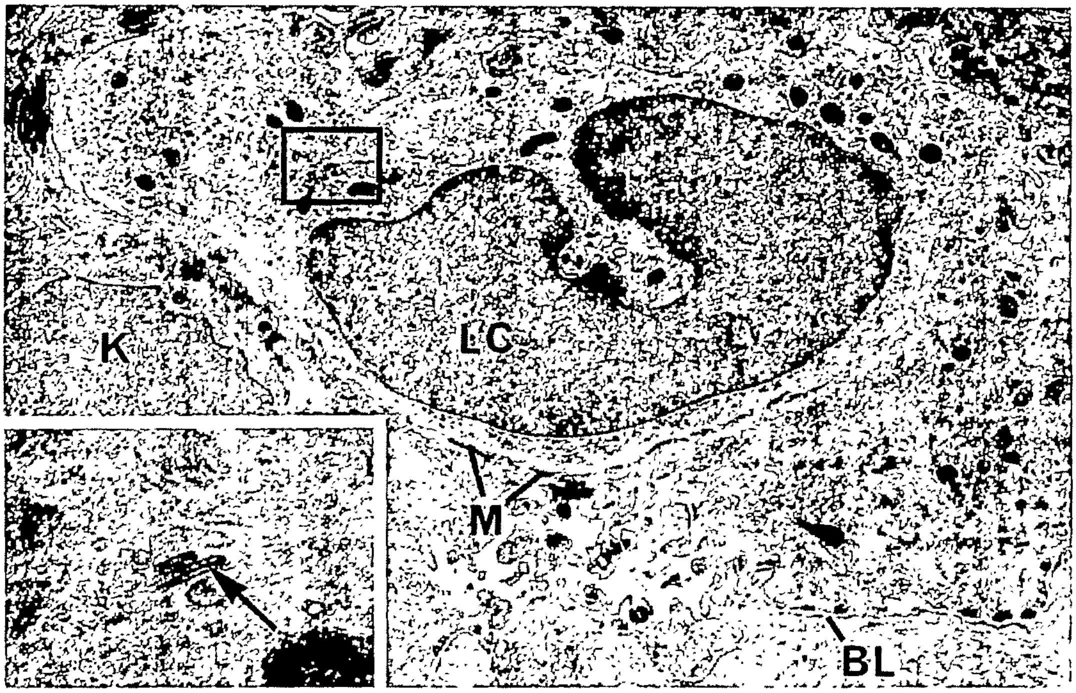

FIGURE 1. A Langerhans cell in the basal layer of the epidermis of a 45-year-old Aboriginal woman prior to exposure to UV. BL, basal lamina; K, keratinocyte; M, material resembling compacted intercellular melanosomes. (Original × 10,000). Inset: A Birbeck granule with a very electron-dense central lamina (arrow). (Original × 36,000).

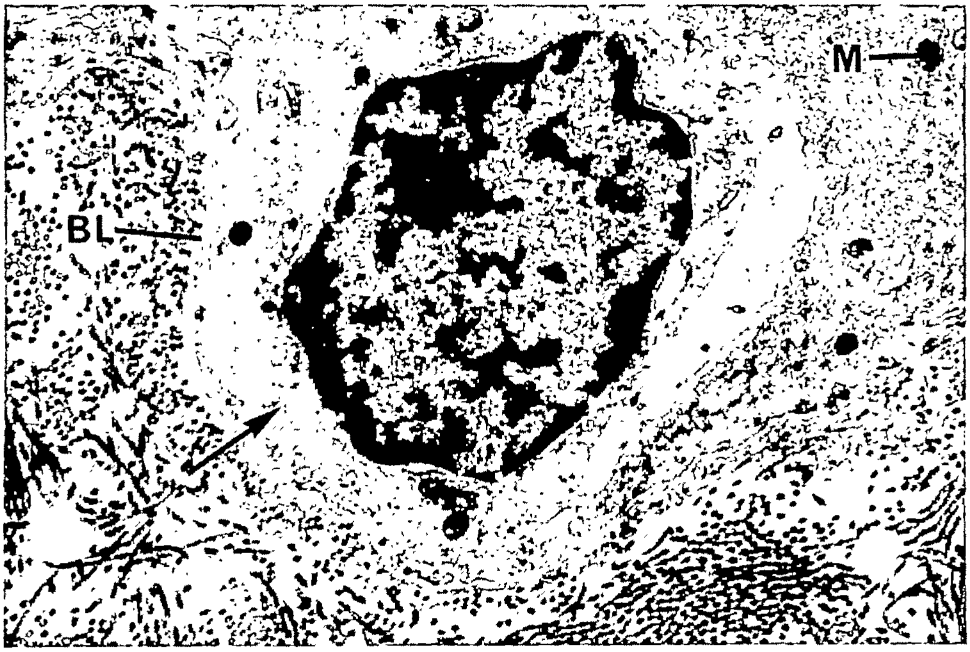

FIGURE 2. Lymphocyte-like cell (arrow) in the basal layer of the epidermis, protruding into the dermis, from a 35-year-old Aboriginal woman prior to UV exposure. BL, basal lamina; M, melanosome in keratinocyte. (Original × 15,700).

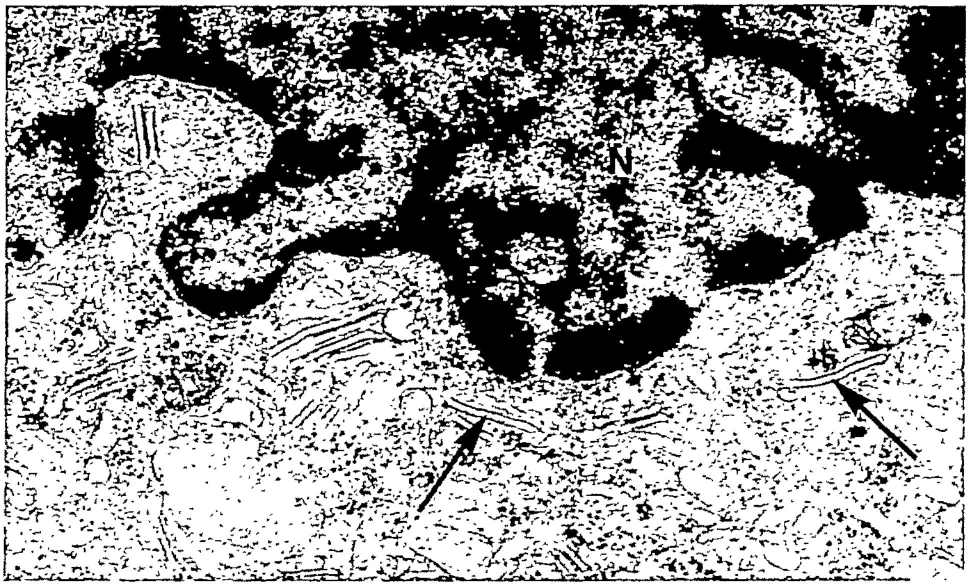

FIGURE 3. A Langerhans cell in the mid epidermis of the same Aboriginal subject as shown in Figure 1, after UV exposure, showing numerous Birbeck granules (arrows) in which the central lamina is not visible. N, nucleus. (Original × 35,000).

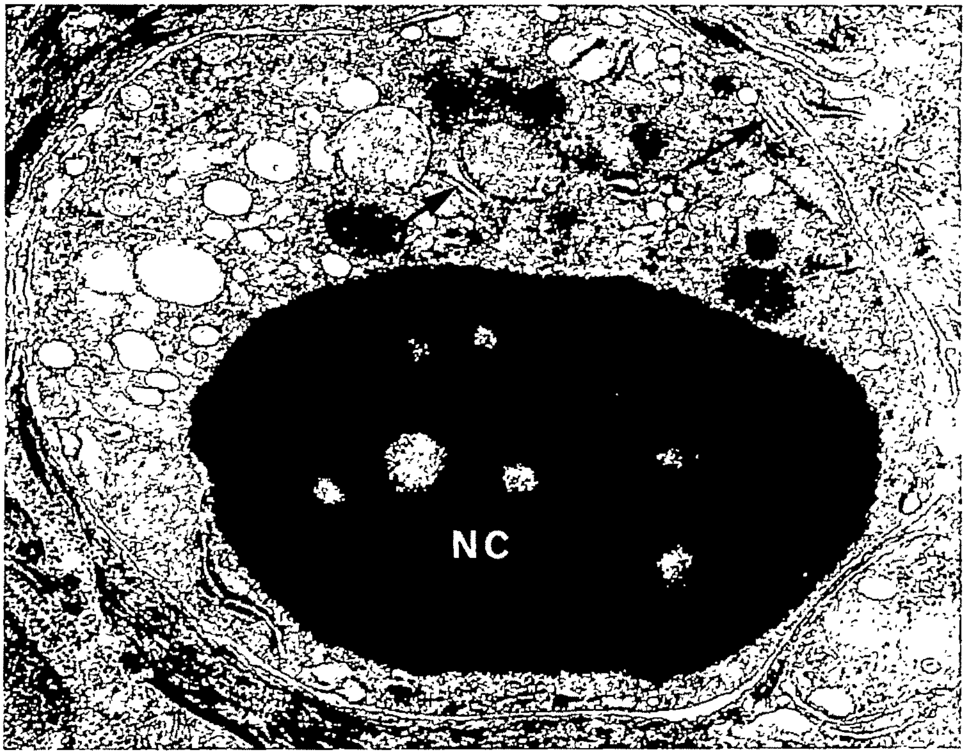

FIGURE 4. A suprabasal LC in an early stage of apoptosis showing condensed nuclear chromatin (NC) and organelles, in the same Aboriginal subject as shown in Figure 1, five days after UV exposure. Arrows indicated Birbeck granules. (Original × 25,000).

increase in melanosome content of the melanocytes and keratinocytes was evident at this time. Lymphocyte-like cells were also more common in the basal layer than they had been in previous samples. No further changes were observed in the keratinocytes.

Celtic subjects

Prior to UV exposure. LC (mean 3·8 ± 0·4/mm) were located at mid (74%) and suprabasal (26%) levels in the epidermis; some dendrites extended to more superficial levels. Their ultrastructure was similar to that usually encountered in Caucasian epidermis, although dilated vesicles of smooth endoplasmic reticulum containing flocculent material were prominent in some cells. The LC contained variable numbers of normal Birbeck granules (mean 10·7 ± 2·9/LC), those cells closest to the basal lamina had fewer than the more superficial cells.

Melanosomes were sparse within the epidermal keratinocytes and negligible within the basal melanocytes (Fig. 5). Lymphocyte-like cells similar to those found in Aboriginal subjects prior to UV exposure were observed in the basal layer.

Two hours after UV exposure. All Celtic subjects developed slight erythema in response to UV

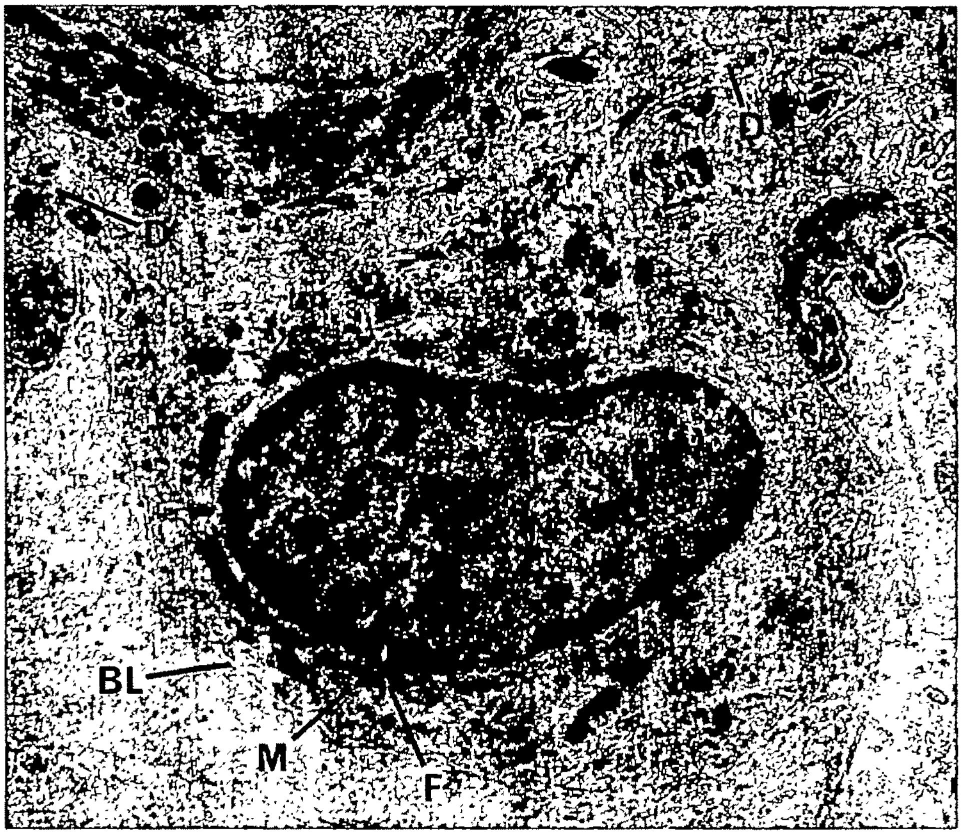

FIGURE 5. A melanocyte in the basal layer of the epidermis in a 36-year-old man of Celtic descent prior to UV exposure, showing marked protrusion of the cell into the dermis, a rounded nucleus (N), sparse melanosomes (M), numerous cytoplasmic filaments (F) and dendrites (D) extending distally between keratinocytes. BL, basal lamina; K, keratinocyte. (Original × 14,500).

exposure. Dendritic LC were now located at lower levels of the epidermis, mostly (75%) in the basal layer. LC appeared to be less numerous (mean 1·3 ± 0·3/mm) and they contained comparatively few Birbeck granules (mean 4·9 ± 0·9/LC). Endoplasmic reticulum and mitochondria in some of the LC were dilated and partially disrupted, indicating cellular damage. Further evidence of cellular disruption was observed in some of the keratinocytes of the spinous layer, which were distorted by large intracytoplasmic vacuoles containing flocculent material of unknown identity (Fig. 6).

Melanocytes in the basal layer of the epidermis were more numerous, as were the melanosomes within and between the keratinocytes. The melanosome content was also increased in some melanocytes, but was unchanged in others. Lymphocyte-like cells were again present in the basal layer of the epidermis; no evidence of cellular damage was observed in these cells.

Five days after UV exposure. The population of epidermal LC continued to appear depleted (mean 2·0 ± 0·9/mm). Most LC were basally located (83%), dendritic, with few Birbeck granules (mean 2·4 ± 0·4/LC) and showed evidence of cellular damage similar to that seen 2 h after UV exposure. Large, dendritic IC were common in the epidermis at basal to mid-levels, and some were seen to be crossing the basal lamina accompanied by individual lymphocyte-like cells. Several such IC/lymphocyte complexes (Fig. 7) as well as LC/lymphocyte complexes, were observed in the basal layer, but no lymphocyte-like cells were seen above this location. The IC had the characteristics of very active cells with the general appearance of LC, but without

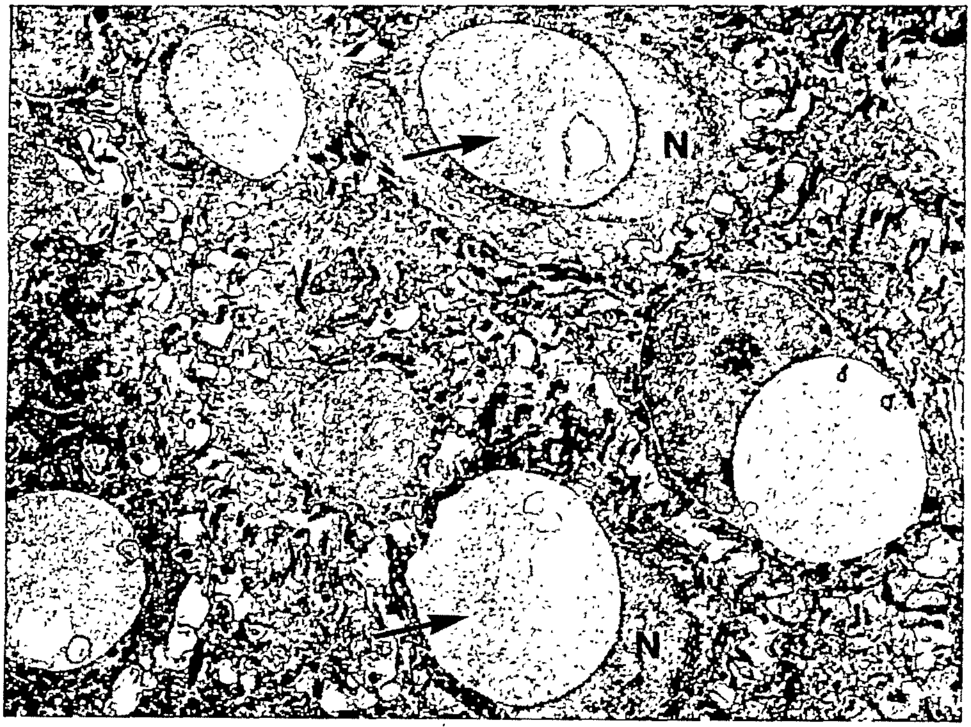

FIGURE 6. The spinous layer of the epidermis of the same Celtic subject as in Figure 5, two hours after UV exposure, showing distortion of keratinocyte nuclei (N) by large intracytoplasmic vacuoles (arrows) containing flocculent material. (Original × 4000).

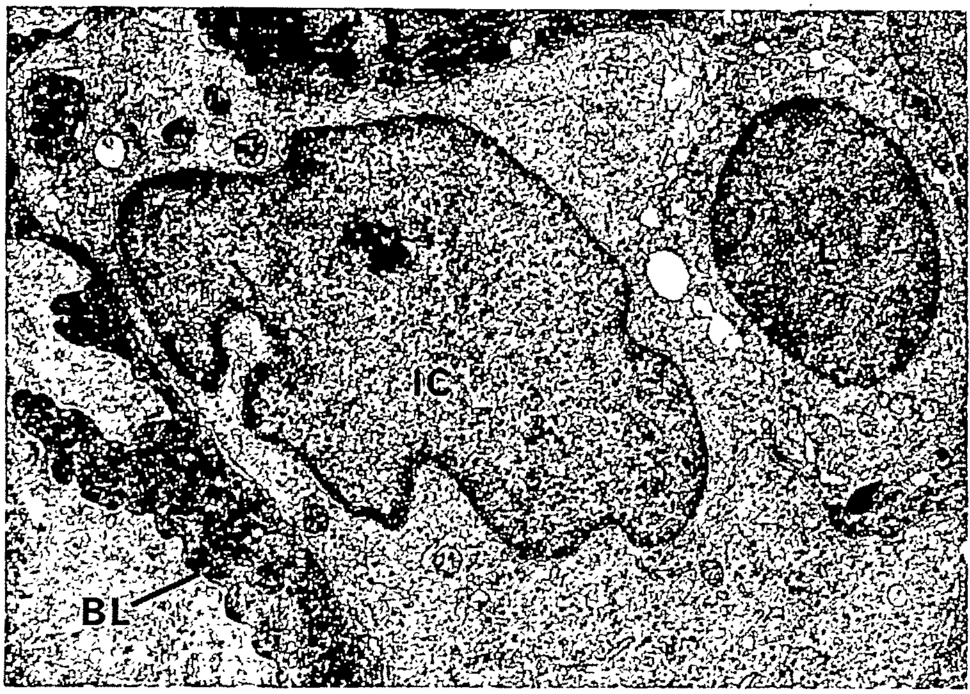

FIGURE 7. An 'indeterminate cell' (IC) in close apposition to a lymphocyte (L) in the basal layer of the epidermis in the same Celtic subject as in Figure 5, five days after UV exposure. BL, basal lamina. (Original × 10,500).

Birbeck granules. No LC were observed in the papillary dermis at this time or in earlier samples. The melanosome content within and between the keratinocytes and melanocytes was increased substantially in all subjects, but was always less than that in Aboriginal epidermis.

DISCUSSION

The responses of epidermal LC and melanocytes in the inherently dark-skinned Aboriginal subjects to small doses of UV differed markedly from those in the fair-skinned Celtic subjects. Epidermal LC, which were mostly located one to two cells above the basal lamina in the Aboriginal subjects became relocated to mid levels following UV exposure and their Birbeck granules appeared to become more numerous. In contrast, LC located at mid-epidermal levels in the Celtic subjects became relocated to the basal layer and seemed to contain fewer Birbeck granules. The number of Birbeck granules in LC of both dark and fair-skinned subjects appeared to depend on the location of the cells within the epidermis. As observed in previous studies,[16,17] Birbeck granules were more numerous in LC in the mid layers of the epidermis than in those in the basal layer. In Aboriginal epidermis the apparent increase in the number of Birbeck granules in LC following UV exposure has been found to coincide with an increase in the intensity of the T6 surface antigen reaction.[8] This suggests that LC may increase their synthesis of T6 antigen in response to low doses of UV. Although functional relationships between the T6 antigen and Birbeck granules have yet to be clearly established, studies on histiocytosis X cells[18] indicate that T6 may be involved in uptake of extracellular substances and that Birbeck granules may represent a specialized structure involved in the internalization or processing of foreign antigens. A similar function, involving receptor-mediated endocytosis, has also been ascribed to LC in normal epidermis treated with anti-T6 antibody[19] in which immuno-gold labelling of the central lamina of Birbeck granules was demonstrated. The central lamina of Birbeck granule-like structures associated with the plasma membranes of these LC was found to be discontinuous. The apparent transient loss of the central lamina from Birbeck granules in LC of Aboriginal subjects following UV exposure may, therefore, indicate a change in the functional state of these cells.

In normal epidermis and dermis, T6 surface antigens are present not only on LC, but also on IC, which are thought to be immature or precursor LC, capable of migrating into the epidermis to replenish the LC population.[20,21] These cells are devoid of Birbeck granules, but this does not necessarily exclude a possible connection between Birbeck granules and T6 antigen, but may merely suggest that Birbeck granules are membrane specializations connected with functions carried out by mature T6+cells.[22] Although cell surface antigens were not studied by immunoelectron microscopy in the present study, it is possible that the IC observed in the Celtic subjects exposed to UV may correspond to the large, dendritic, strongly DR+ cells observed previously by light microscopy in these subjects, which it was suggested were resistant suppressor cells.[8] The intimate epidermal association of LC and IC with lymphocytes observed in the present and previous studies[23] suggests functional interactions between these cell types. The nature of these relationships requires further study using immunoelectron microscopy.

The observed depletion of LC in both Aboriginal and Celtic subjects following UV exposure, although based on comparatively small numbers of cells and small groups of subjects, is in agreement with our previous light microscopic study[8] conducted on the same groups of subjects and under the same experimental conditions. This study showed that the protection given to LC by darkly pigmented Aboriginal epidermis, against UV induced effects, although greater than that in fair Celtic skin, was insufficient to prevent transient depletion of these cells. The results of the present study indicate that LC depletion in the two racial groups may have occurred through different processes. Apart from loss of LC probably by migration out of the epidermis following UV exposure,[23,24] LC depletion occurred in the Aboriginal subjects by apoptosis, whereas the organelle and membrane disruption displayed by some of the LC in the Celtic subjects suggests that part of their depletion may have resulted from direct cellular damage. Apoptosis is an active process of organised death of scattered cells which commonly occurs during the physiological atrophy of tissues.[25] This process also occurs in cell-mediated immune responses in the epidermis, in disorders such as lichen planus, and in human epidermis following UV exposure[26] or sunburn[27] in which epidermal keratinocytes undergoing apoptosis have been termed 'sunburn cells'. These cells were not observed in the present study in which relatively small doses of UV were given. However, LC undergoing apoptosis, which have not been identified previously, were observed in the Aboriginal subjects following UV exposure. Phagocytosis by neighbouring keratinocytes of a few LC exhibiting cytoplasmic and nuclear condensation and fragmentation has been described in human epidermis of unspecified racial origin following exposure to UV.[28] All of these features are typical characteristics of apoptosis.[25] The extent of LC depletion may have been more extensive than suggested by the few apoptotic LC observed in the present study, as the formation and dissolution of apoptotic cells and bodies is very rapid.[25]

The intracytoplasmic vacuoles observed in the keratinocytes of the spinous layer of the epidermis in the Celtic subjects following UV exposure closely resembled those described previously in Caucasian epidermis following UV irradiation, which were interpreted as being indicative of acclerated metabolic activity rather than cellular degeneration.[29]

The UV-induced degenerative changes in LC ultrastructure in the Celtic subjects and the disruption of their antigenic surface markers observed previously[8] suggests a loss or suppression of immunological function of these cells, whereas the absence of these changes in the LC of Aboriginal subjects and the enhancement of their T6 response to UV[8] indicates retention or stimulation of immunological function. These opposite responses of LC in Celtic and Aboriginal subjects exposed to UV may explain the very high incidence of skin cancers in the former and their relative rarity in the latter.

Epidermal melanocytes in the Celtic subjects were largely inactive and few in number prior to irradiation, and responded to UV exposure with slight increases in melanosome formation in some subjects immediately following irradiation, followed by more substantial increases in all subjects by 5 days after irradiation. Conversely, the melanocytes in the Aboriginal subjects were moderately active and more numerous prior to irradiation, and responded with a substantial initial increase in melanosome formation followed by a further, but only slight increase by 5 days after irradiation. These contrasting responses suggest that the epidermal melanocytes in the Aboriginal subjects responded more efficiently and were more sensitive to UV than those in the Celtic subjects.

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Histopathological and immunohistochemical study of amyloidosis cutis nodularis atrophicans— Comparison with systemic amyloidosis

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SUMMARY

Three cases of amyloidosis cutis nodularis atrophicans (ACNA) were investigated histologically and immunohistochemically to determine the nature and origin of the deposited amyloid. A pulmonary lesion from a case of nodular pulmonary amyloidosis, and cutaneous lesions from three cases of primary systemic amyloidosis, two cases of secondary systemic amyloidosis and three cases of secondary cutaneous amyloidosis following basal cell epithelioma were also examined for comparison.

Histology showed infiltration of numerous plasma cells adjacent to amyloid deposits in ACNA and nodular pulmonary amyloidosis, but not in systemic amyloidosis. Immunohistochemically, the cytoplasm of the plasma cells was stained with anti-immunoglobulin light chain or anti-Bence-Jones protein antisera or both, and amyloid material stained with anti-AL antiserum in ACNA and nodular pulmonary amyloidosis. These results suggest that, in ACNA, the plasma cells may produce and secrete immunoglobulin light chains or Bence-Jones protein or both, which undergo proteolysis to protein AL or amyloid fibril proteins which have the same immunoglobulin determinants as protein AL. The product is then deposited locally to form nodules in the dermis.

Amyloidosis cutis nodularis atrophicans (ACNA) is a very rare disease, first described by Gottron,[1] and there have been only 13 cases reported in Japan, to our knowledge. The disease has been classified as a primary localized cutaneous amyloidosis, but recently there has been controversy as to whether ACNA is systemic.

We have reported a case of ACNA in which the amyloid deposits and plasma cells stained with anti-AL (λ type) antiserum.[2,3]

In the present study we have compared ACNA histopathologically and immunohistochemically with other types of amyloidosis, both systemic and secondary.

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METHODS

Biopsy specimens were obtained from three cases of ACNA, one case of nodular pulmonary amyloidosis (NPA), three cases of primary systemic amyloidosis (PSA) (cutaneous lesions), two cases of secondary systemic amyloidosis (SSA) (cutaneous lesions), and three cases of secondary cutaneous amyloidosis (SCA) following basal cell epithelioma. Clinical details are given in Table 1, and the appearance of ACNA lesions is illustrated in Figure 1.

The specimens were examined histologically using haematoxylin and eosin, thioflavin T stain, dylon stain[8] and Congo red stain before and after exposure to potassium permanganate.[9,10] Immunohistochemistry was carried out by the peroxidase-antiperoxidase method, using anti-IgG, IgA, IgM, immunoglobulin light chain, Bence-Jones protein, prealbumin, protein AL (κ and λ types)[11] and protein AA (amyloid fibril protein A)[12] antisera. Controls were carried out using normal rabbit serum as the primary antiserum.

TABLE 1. Clinical data of patients studied

Case no.	Diagnosis	Age (years)	Sex	Complications	Serum M protein	Urinary Bence-Jones protein	Rectal amyloid	Other details
1[2,3]	ACNA	60	F	—	–	–	ND	RA(+)
2[4]	ACNA	51	F	Sjögren's syndrome	–	–	–	–
3[5]	ACNA	43	F	Sjögren's syndrome	–	–	–	–
4[6]	NPA	70	M	—	ND	–	ND	–
5	PSA	74	M	—	+	+(λ)	+	nodules
6	PSA	59	M	—	+IgG(λ)	ND	+	ecchymosis
7[7]	PSA	69	M	Multiple myeloma	+IgA(λ)	+(λ)	+	bullae
8	SSA	86	M	Colonic carcinoma	ND	ND	+	nodules
9*	SSA	58	F	Pulmonary tuberculosis	–	–	–	nodules
10	SCA	78	F	Basal cell epithelioma	ND	ND	ND	—
11	SCA	74	M	Basal cell epithelioma	ND	ND	ND	—
12	SCA	86	F	Basal cell epithelioma	ND	ND	ND	—

ND: not done

* This case was presented at the 38th annual meeting of the Japanese Dermatological Society, Western Branch, Saga, 1986.

ACNA: Amyloidosis cutis nodularis atrophicans; NPA: nodular pulmonary amyloidosis; PSA; primary systemic amyloidosis; SSA: secondary systemic amyloidosis; SCA: secondary cutaneous amyloidosis.

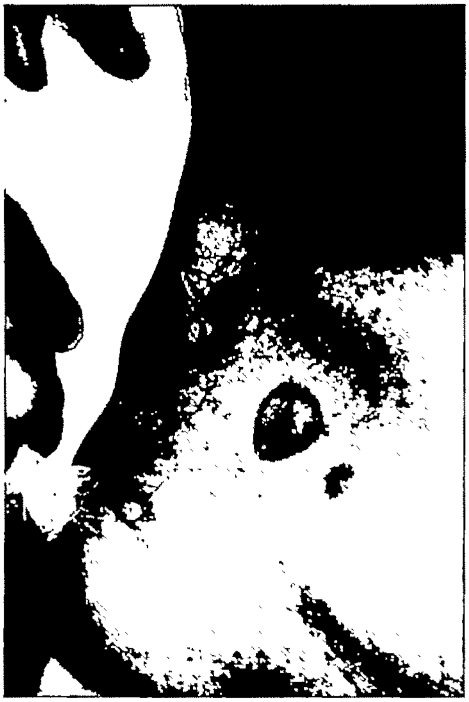
FIGURE 1. Clinical appearance of Case 1. Four nodules were present on the genitocrural area.

FIGURE 2. Histology of Case 1. A mass of amorphous material (arrow) was seen with patchy plasma cell infiltration (double arrow). (H & E, original × 40).

FIGURE 3. A cutaneous lesion of primary systemic amyloidosis (Case 5). Nodular amorphous amyloid material (arrow) was seen without plasma cell infiltration. (Alkaline Congo red, original × 80).

FIGURE 4. A cutaneous lesion of secondary systemic amyloidosis (Case 8). No infiltration of plasma cells was seen. Arrow indicates amyloid deposits. (Alkaline Congo red, original × 80).

(1)



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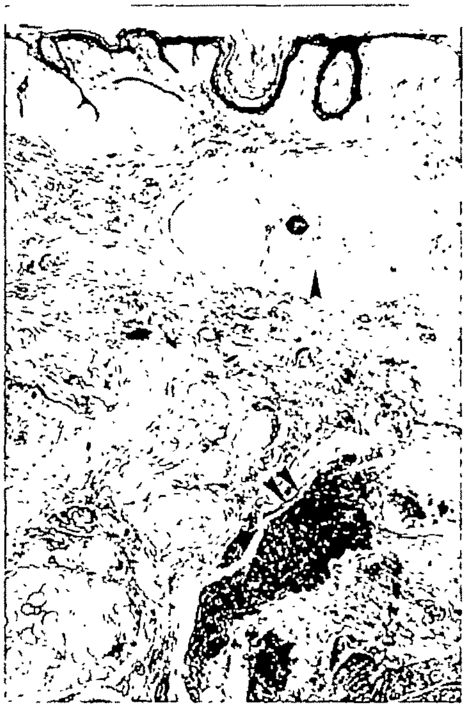

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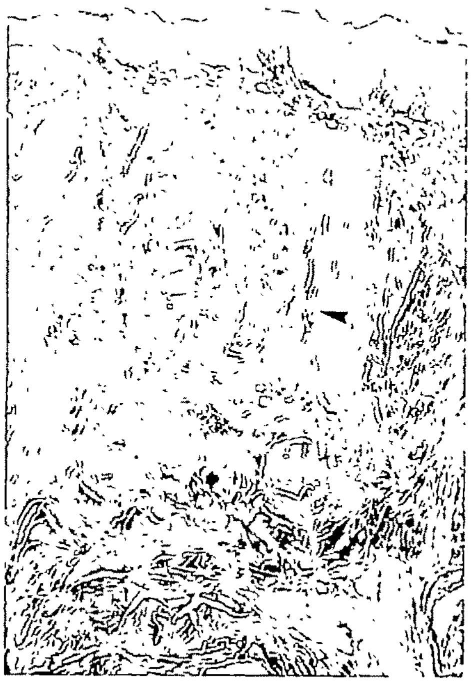

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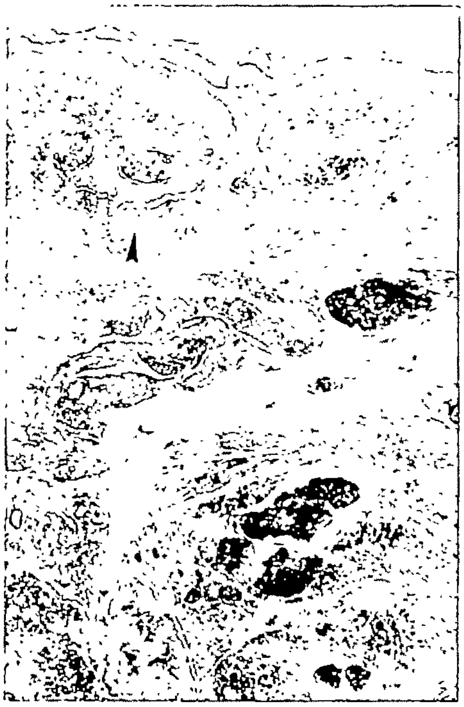

RESULTS

Histology

The results are summarized in Table 2. The haematoxylin and eosin stained specimen from Case 1 showed an atrophic epidermis, band-like deposits of eosinophilic amorphous material in the papillary and subpapillary dermis, doughnut shaped deposits around blood vessels and dermal appendages, and massive deposits in the dermis and subcutis. Focal infiltrates of plasma cells, lymphocytes and histiocytes were seen around the deposited material. Some of the histiocytes were seen ingesting the amorphous material (Fig. 2). The eosinophilic material stained crimson with Congo red and had green birefringence, and did not lose its stainability after exposure to potassium permanganate. It stained with dylon and thioflavin T, and thus was identified as amyloid. Cases 2 and 3 showed similar appearances, but Case 3 did not have the massive amyloid deposits in the mid and deep dermis.

Case 4 (NPA) showed massive amounts of amorphous material deposited in the walls of the alveoli, arterioles and venules. Numerous infiltrates of lymphocytes and plasma cells were seen. The amorphous material had the staining characteristics of amyloid.

Case 5 (PSA) showed amorphous material deposited in a band-like pattern in the papillary dermis, a massive deposit in the deep dermis, and a doughnut shaped pattern of deposition around blood vessels and skin appendages. Few cell infiltrates were seen and plasma cells were not found. The amorphous material showed the staining characteristics of amyloid (Fig. 3). Case 6 (PSA) showed similar results, but there were no massive deposits of amorphous material in the mid and deep dermis and the amyloid material stained weakly and unevenly with Congo red. Case 7 (PSA) also showed similar results to Case 5, but there were no massive deposits of amorphous material in the mid and deep dermis.

Case 8 (SSA) showed deposits of amorphous material in the mid and deep dermis and around

TABLE 2. Histological results

		Amyloid deposits					Congo red
	Case no.	In papillary dermis	In mid and deep dermis	Around vessels and skin appendages	In rectal mucosa	Plasma cells	after exposure to potassium permanganate
ACNA	1	+	+	+	ND	+	resistant
	2	+	+	+	−	+	resistant
	3	+	−	+	−	+	resistant
NPA	4	+ (nodular in lung tissue)		+	ND	+	resistant
PSA	5	+	+	+	+	−	resistant
	6	+	−	+	+	−	resistant
	7	+	−	+	+	−	resistant
SSA	8	−	+	+	+	±	sensitive
	9	−	+	+	−	−	sensitive
SCA	10	+ (patchy adjacent to the tumour nests)			ND	+	resistant
	11				ND	+	resistant
	12				ND	+	resistant

ND: not done

blood vessels and dermal appendages. Slight infiltration of lymphocytes and plasma cells was seen. The amorphous material stained evenly with dylon, thioflavin T and alkaline Congo red (Fig. 4), but did not stain after exposure to potassium permanganate. Case 9 (SSA) showed similar results to Case 8, but cell infiltration was sparser. The amorphous material was identified as protein AA.

Case 10 (SCA) showed many punctate collections of amorphous material adjacent to the tumour nests of basal cell epithelioma. Massive lymphocyte and plasma cell infiltrates were seen around the tumour nests. The amorphous material showed the staining characteristics of amyloid. Cases 11 and 12 (SCA) gave very similar results.

Immunohistochemistry

The results are summarized in Table 3. In Case 1, the cytoplasm of infiltrating plasma cells stained with anti-IgG, IgA, immunoglobulin light chain (κ and λ types), and Bence-Jones protein (κ and λ types) antisera, and especially intensely with anti-immunoglobulin light chain λ type and Bence-Jones protein λ type antisera (Fig. 5). The protein AL λ type antiserum stained both the cytoplasm of the plasma cells and the amyloid material intensely (Fig. 6), but anti-AL κ type antiserum failed to stain either of these. In Case 2, anti-immunoglobulin light chain (κ and λ types) and Bence-Jones protein (κ and λ types) antisera stained both the cytoplasm of the

TABLE 3. Immunohistochemistry

	Case no.		Antibodies										
			IgG	IgA	IgM	L(κ)	L(λ)	B-J(κ)	B-J(λ)	Prealbumin	AL(κ)	AL(λ)	AA
ACNA	1	amyloid material	−	−	−	−	−	−	−	−	−	++	−
		plasma cells	+	+	−	+	++	+	++	−	−	++	−
	2	amyloid material	+*	+*	−	+	++	+	++	ND	±	±	−
		plasma cells	+*	+*	−	+	++	+	++	ND	−	±	−
	3	amyloid material	ND	ND	ND	ND	ND	+	−	ND	+	−	−
		plasma cells	ND	ND	ND	ND	ND	++	±	ND	−	−	−
NPA	4	amyloid material	ND	ND	ND	ND	ND	±	±	−	−	+	−
		plasma cells	ND	ND	ND	ND	ND	+	+	−	−	+	−
PSA	5	amyloid material	−	−	−	−	+	−	+	−	−	−	−
	6	amyloid material	±	±	±	−	+	−	+	−	−	+	−
	7	amyloid material	±	±	−	−	−	−	−	−	−	−	−
SSA	8	amyloid material	−	−	−	−	−	±	−	−	−	−	−
	9	amyloid material	−	−	−	−	−	−	−	ND	ND	ND	−
SCA	10	amyloid material	+	−	−	−	−	−	−	−	−	−	ND
		plasma cells	+	+	−	−	−	−	−	−	−	−	ND
	11	amyloid material	+	−	−	−	−	−	−	ND	−	−	ND
		plasma cells	+	+	−	−	−	−	−	ND	−	−	ND
	12	amyloid material	+	ND	ND	−	−	−	−	ND	−	−	ND
		plasma cells	+	ND	ND	−	−	−	−	ND	−	−	ND

* confirmed by immunofluorescence. ND: not done

−: not stained; ± weakly stained; +: moderately stained; ++: intensely stained

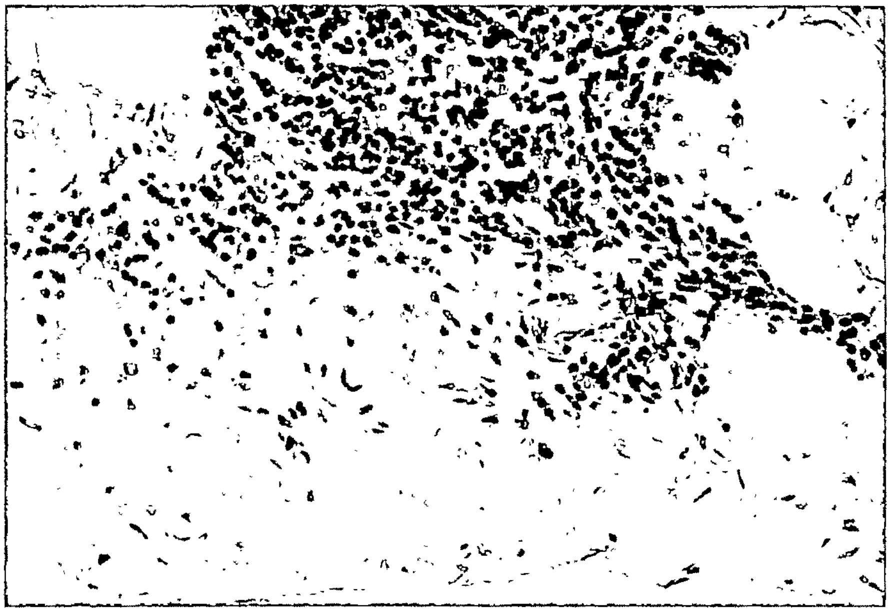

FIGURE 5. Immunohistochemistry of a lesion of ACNA (Case 1). The infiltrating plasma cells are stained with anti-Bence-Jones protein (λ type) antibody. (PAP method, original × 200).

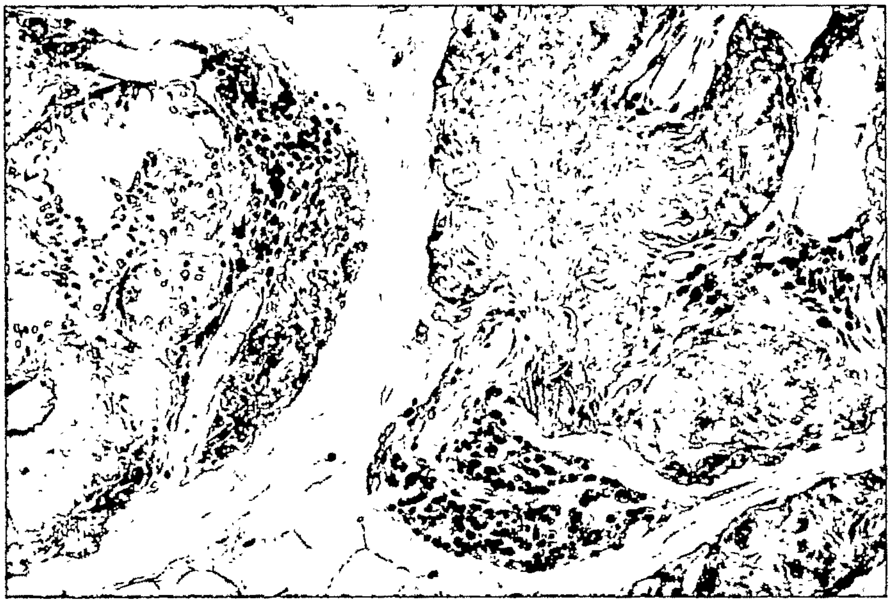

FIGURE 6. A lesion of ACNA (Case 1) stained with anti-AL λ type antibody. Both the amyloid material and the plasma cells are stained. (PAP method, original × 200).

plasma cells and the amyloid material, and λ type antisera stained these especially intensely. Anti-protein AL (κ and λ types) antisera stained the amyloid material weakly, and anti-protein AL (λ type) antiserum stained the cytoplasm of the plasma cells weakly. In Case 3, anti-Bence-Jones protein (κ type) antiserum stained the cytoplasm of infiltrating plasma cells intensely and the amyloid material was also stained. Anti-AL (κ type) antiserum also stained the amyloid material.

These results indicate that in ACNA, infiltrating plasma cells contain immunoglobulin light chains (κ and λ types) and Bence-Jones protein (κ and λ types), and the amyloid material contains protein AL, or amyloid fibrils which have the same antigenicity as protein AL.

In Case 4 (NPA), anti-Bence-Jones protein (κ and λ types) antisera stained the cytoplasm of the plasma cells, and the amyloid material stained weakly. Anti-AL (λ type) antiserum stained both the cytoplasm of plasma cells and the amyloid material. These results indicate that in NPA the infiltrating plasma cells contain Bence-Jones protein, and the amyloid material contains protein AL λ type or amyloid fibrils which have the same antigenicity as protein AL.

In Case 5 (PSA), the amyloid material stained with anti-immunoglobulin light chain λ type and Bence-Jones protein λ type antisera, but was negative with anti-protein AL (κ and λ types), IgG, IgA, IgM, light chain κ type and Bence-Jones protein κ type antisera. In Case 6 (PSA), the amyloid material stained with anti-immunoglobulin light chain λ type, Bence-Jones protein λ type, protein AL λ type antisera, and weakly with anti-IgG, IgA and IgM antisera. In Case 7 (PSA) the amyloid material stained weakly with anti-IgG and IgA antisera only. These cases were definitely diagnosed as PSA on the basis of clinical and laboratory investigations. The failure of the anti-AL antiserum to stain the amyloid material in Cases 5 and 7 may be due to a difference in the subgroups of the light chain variable region.[10]

In Case 8 (SSA), the amyloid material stained weakly with anti-Bence-Jones protein κ type antiserum only; there was no staining with the other antisera including anti-protein AA antiserum. In Case 9, the amyloid material failed to stain with any of the antisera tested. The amorphous material in these two cases was identified histochemically as protein AA. The reasons for the failure of anti-AA antiserum to stain this material were not clear.

In Cases 10, 11 and 12 (SCA) the amyloid material and the cytoplasm of the plasma cells stained with anti-IgG antiserum, but was negative with anti-light chain (κ and λ types), Bence-Jones protein (κ and λ types) and protein AL (κ and λ types) antisera. The cytoplasm of the plasma cells stained with anti-IgA antiserum in Cases 10 and 11. These plasma cells were thought to have nothing to do with the amyloid deposits.

DISCUSSION

Since the first report of ACNA by Gottron,[1] there have been about 20 case reports in the world literature and 13 cases in Japan. The disease has been classified as a primary localized cutaneous amyloidosis, but recently there has been some controversy as to whether the disease is a localized amyloidosis or a systemic one[5,13–16] since the amyloid deposits in ACNA are situated deep in the dermis and around skin appendages as well as in the subpapillary region, ACNA is sometimes accompanied by Sjögren's syndrome,[4,5,13,14] and in one case amyloid deposits were found in the rectal mucosa.[15]

In the present study we found, in both ACNA and NPA, infiltrating plasma cells containing immunoglobulin light chains (κ and λ types) and Bence-Jones protein (κ and λ types), and amyloid material containing protein AL (κ or λ types or both) or amyloid fibrils having the same antigenic determinants as this protein.

From these results in ACNA and NPA, it appears that plasma cells produce and secrete immunoglobulin light chains or Bence-Jones protein or both. The secreted protein appears then to be phagocytosed by macrophages or histiocytes, degraded into protein AL, or amyloid fibrils which have the same antigenic determinants as protein AL, and deposited in the skin or other tissues to form papules or nodules.

The diagnosis of systemic amyloidosis is made when the deposited amyloid is proved to contain protein AL. However, Sugawara *et al.*[17] reported that using the PAP method the amyloid material in ACNA showed a positive reaction with anti-protein AL λ type antiserum, and attributed the deposition of amyloid to the plasma cell dyscrasia in the affected area. We have also found immunoglobulin light chain (κ and λ types), Bence-Jones protein (κ and λ types), IgG and IgA in the cytoplasm of plasma cells, and protein AL (λ type) in the amyloid material in ACNA, and suggested that the localized amyloidosis had a protein AL origin.[2,3]

Northcutt *et al.*[18] reported a case of nodular cutaneous amyloidosis of the genital area with neither circulating M protein and Bence-Jones protein nor rectal amyloid deposits. They found that deposited amyloid and the cytoplasm of infiltrating plasma cells showed a strong positive reaction with anti-light chain κ type, a weakly positive reaction with λ type, and positive reactions with anti-IgG, IgA and IgM antisera, and postulated that the locally proliferating plasma cells secreted immunoglobulin light chains containing fibrilogenic amino acids, which then were phagocytosed by macrophages, proteolysed, and yielded insoluble fibrils having a β-pleated sheet configuration.

Page *et al.*[19] reported a case of localized nodular pulmonary amyloidosis which they determined biochemically to have originated from Bence-Jones protein λ type. The patient had no circulating M protein and the lesions were infiltrated with plasma cells. The authors considered that the nodular collections of amyloid were actually plasma cell lesions in which the immunoglobulin produced by the cells had been modified, perhaps by proteolysis, to form insoluble fibrils.

Fujihara *et al.*[20] demonstrated immunohistochemically the presence of amyloid fibrils having the same antigenicity as protein AL λ type in nine cases, and the infiltration of plasma cells in juxtaposition to amyloid material in 10 cases, out of 11 patients with primary localized genito-urinary amyloidosis, which was consistent with the results of Page *et al.*[19]

Glenner stated in his review[21] that the cellular source of the amyloid fibrils of immunoglobulin origin was probably an immunocyte-derived clone, whose synthetic products were either locally deposited or disseminated by the blood stream.

From these results ACNA, like NPA or genito-urinary amyloidosis, is probably a localized form of amyloidosis of protein AL origin.

The 13 cases of ACNA reported in Japan are listed in Table 4. In seven cases amyloid deposits were proven immunohistochemically or biochemically to have originated from protein AL or amyloid fibrils having the same antigenicity as protein AL. No case had circulating Bence-Jones protein or M protein except one (Case 13) who had benign M proteinaemia. Plasma cell infiltration was seen in 10 cases. There is a possibility that 3 cases without plasma cell infiltration were in the early stages of systemic amyloidosis, since one case (Case 2) had ecchymosis and another (Case 3) had a high serum IgG level (3640 mg/dl).

Whether ACNA and NPA are polyclonal (reactive) diseases or monoclonal (probably neoplastic) remains unanswered.

In our Cases 1 and 3 the disease appeared to be monoclonal, but in Cases 2 and 4 it appeared to be polyclonal, on the basis of our present results. The fact that patients with ACNA rarely develop systemic amyloidosis in the long-term supports the conclusion that ACNA is a reactive

TABLE 4. Details of ACNA cases reported in Japan

No.	Source	Age (years)	Sex	Site of lesion	Complications	Amyloid in dermis	Amyloid around vessels	Plasma cells	M protein	Bence-Jones protein	Rectal amyloid	Other remarks
1	Nakamura[22]	36	F	thighs	—	+	+	+	–	–	ND	none
2	Kawashima[23]	33	F	trunk	—	+	+	–	–	–	–	bleeding
3	Yamazaki[13]	53	F	trunk, thighs	Sjögren's syndrome	+	+	–	–	–	ND	γ-globulin 30·5%, IgG 1:3640
4	Ootsuki[24]	64	F	forearm	—	+	+	–	–	–	ND	none
5	Hada[5]	43	F	thighs, abdomen	Sjögren's syndrome	–	+	+	–	–	–	AL(κ) (+)*
6	Yamazaki[25]	76	M	forearm, trunk	lung carcinoma	+	+	+	–	–	ND	γ-globulin 39·6%
7	Imai[26,28]	61	F	trunk, thighs	—	+	+	+	–	–	ND	AL(λ) (+)*
8	Sugawara[17]	68	F	all over the body	—	+	+	+	–	–	ND	AL(λ) (+)*
9	Aoki[27]	63	F	abdomen, thighs	—	+	+	+	–	–	–	AL(λ) (–)*
10	Masuda[2,3]	60	F	genitocrural	—	+	+	+	–	–	ND	AL(λ) (+)*
11	Inagaki[4]	51	F	trunk	Sjögren's syndrome	+	+	+	–	–	–	AL(κ, λ) (±)*
12	Muramatsu[16]	60	F	genital	—	+	+	+	–	–	ND	AL(λ) (+)*
13	Aoki[14]	63	F	trunk	Sjögren's syndrome	+	+	+	+	–	–	AL(λ) (+)*

* nature of amyloid deposit. ND: not done.

disease. ACNA patients with deposition of amyloid in the rectal mucosa or with circulating monoclonal IgG (λ type) may go on to develop systemic amyloidosis.

The characteristic atrophic features of the disease may be the result of granulomatous tissue phagocytosing the amyloid material, as seen in one of our cases (Case 1).

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Carbon monoxide poisoning: Optics and histology of skin and blood

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SUMMARY

In 10 fatal cases of carbon monoxide poisoning an optical study of the skin colour was made by reflectance spectrophotometry, and the values converted to their visual equivalents. Several circumstances contribute to the difficulty of identifying the cherry-red colour in the skin, such as a low CO concentration, skin pigmentation, washing out of a previously high concentration of CO, and deep venous dilatation with superficial vasconstriction producing the impression of cyanosis. The colour of the altered blood depends on the way the red cells are massed together, their depth below the surface, and the brightness of the background against which they are viewed. These phenomena were compared with the dichroism of oxyhaemoglobin. Damage to eccrine sweat acini was noted early in CO-poisoning. It was probably intensified, in those surviving longer, at skin sites of intermittent pressure anoxia.

Fatal carbon monoxide poisoning in man and animals produces blood of a bright scarlet colour in the victim, which is more striking in appearance than the red tint of arterial blood. In the middle of the 19th century this colour change was already known, and correctly attributed to a stable combination of haemoglobin with carbon monoxide. Claude Bernard and F. Hoppe-Seyler were the great pioneers in this field.[1]

During life and after death the same tint may be detected in the skin, but its presence and recognition may be matters of dispute. In a bulky mass of blood, for instance, the colour differences between oxyhaemoglobin (HbO_2) and carboxyhaemoglobin (HbCO) are not discernible, but when viewed in thin layers, after dilution, or on a white fibrous background such as connective tissue, or on filter paper, the colour contrast may be more apparent. In these circumstances oxyhaemoglobin loses its red colour and becomes yellow, while carboxyhaemoglobin remains crimson. For many years John Scott Haldane[2] had actually used this carmine-like hue on dilution to quantify carbon monoxide uptake in the blood.

The absorption bands and extinction coefficients of oxyhaemoglobin and carboxyhaemoglobin lie close to one another on the visible spectrum. However, the observed colour differences between them are generated principally by the remainder of the spectrum which changes in different ways as the concentration alters. This colour shift without change in composition comprises the phenomenon of 'dichroism'.[3]

The visual impact of a coloured surface upon a normal eye can be derived by calculation from the reflectance spectrum of that surface. In the present study this method was applied in fatal cases of carbon monoxide poisoning and correlated with the colour production by the relevant components in the skin.

METHODS

Reflectance spectra

Reflectance spectra (400–700 nm) were recorded by a fibre-optic spectrophotometer with an 8 mm sensing aperture. The apparatus was designed and constructed at the National Physical Laboratory (Dr R. Turner).

Visual matching

Visual matchings of the recorded spectra for a normal eye were calculated with the aid of a computer programme. Thirty one percentage values, each 10 nm apart, were taken from the reflectance spectra between 400 and 700 nm. Sunlight and diffuse daylight were taken as the two standard light sources. The colour effect was determined from the data provided by the C.I.E. Tristimulus System.[4,5] By this method the colour seen is matched by a single wavelength (the dominant wavelength) sometimes represented as λ_d, but given here in nm. It is modified only by its dilution with white light, expressed as its calculated colour purity, P, and its brightness (or dimness), B, corresponding to the flow of energy acting on the eye.

Reflectance spectra for red blood cells

Red cell aggregates in unclotted normal whole blood were treated with oxygen, dithionite or pure carbon monoxide (kindly provided by Dr D.L.Morgan, Fuel Research Institute) to produce HbO_2, Hb and HbCO. These preparations were soaked and diluted on blotting paper or filter paper, and the reflectance spectra drawn at room temperature while the resulting blots were still moist.

Cases of carbon monoxide poisoning

Ten fatal cases of carbon monoxide poisoning were studied. Three were accidental deaths (burners used in a confined space) and seven were apparently suicides (from motor exhaust fumes). One of the latter showed overwhelming meprobamate poisoning. Blood levels of carbon monoxide quoted are those supplied by the State Medicolegal Laboratories. Strips of livid or discoloured skin from the cadavers were promptly used for obtaining reflectance spectra, and then fixed and processed for histology. The obscuring effect of melanin was reduced by selecting only Caucasian subjects for the study. Notching of the reflectance spectra at approximately 418, 540 and 570 nm suggested HbCO, and 415, 540 and 576 nm were taken as typical for HbO_2.

RESULTS

Colour differences on dilution

Transmission spectrophotometry of diluted HbO_2 and HbCO differed mainly from 420 to 620 nm, between which HbO_2 transmitted more. For HbO_2 the dominant wavelength was 586 nm (yellow-orange), brightness 67%, and purity 36%, while HbCO had a dominant wavelength of 599 nm (reddish orange), a brightness of 44%, and a purity of 50%.

The transmitted colour of carboxyhaemoglobin is, therefore, purer richer and redder than oxyhaemoglobin on equal dilution.

Comparison of reflectance

From diluted blot preparations of HbO_2, Hb (reduced) and HbCO, the reflectance curves allowed the calculation of brightness, purity and dominant wavelength (Table 1). The dominant wavelength decreased with increasing dilution and increasing brightness. Carboxyhaemoglobin tended to remain pink on dilution.

The calculated purity of the colour cannot be taken as a function of the blot thickness, since in the blot system the light path is bidirectional. It represents the effects of transmission inward and dispersion in the reverse direction. A closer guide to the effective density of the blot is therefore its brightness. The colour locus as represented in Figure 1 is intended to illustrate the relationships between colour and dilution as seen with these three coloured compounds. In this diagram the colour locus with differing blood film thicknesses containing Hb, HbO_2 and HbCO is given. The colour seen, i.e. the dominant wavelength, is read off from a straight line from the light source B though the colour locus of the haemoglobin derivative at its supposed dilution point, projected to the edge of the chromaticity diagram along the spectral line.

Connective tissue reflectance

In the intact skin the colour produced by the blood pigments is 'diluted' by the paler connective tissue, much as happens with blood smears soaked into white filter paper. The dominant wavelengths of a variety of connective tissues was determined and the results are shown in Table 2.

The purity of the colour was about 20%, i.e. there was more white than yellow in the hue. Even if elastic tissue contributes a yellowness to the aorta and to certain ligaments, it seemed to us that this colour trend could arise more probably from the presence of unobserved blood.

TABLE 1. The reflectance colour shift of oxyhaemoglobin, haemoglobin and carboxyhaemoglobin on dilution, as shown by the dominant wavelength and by brightness (B) and colour purity (P)

Oxyhaemoglobin			Haemoglobin (red)			Carboxyhaemoglobin		
B %	Dominant wavelength nm	P %	B %	Dominant wavelength nm	P %	B %	Dominant wavelength nm	P %
15	623	36	11	628	11	14	624	19
18	620	38	13	629	16	16	622	21
21	615	37	23	607	24	17	617	33
37	606	38	36	597	25	19	614	38
60	596	22	50	589	14	24	614	32
65	588	33				40	611	27
85	581	19				72	602	7

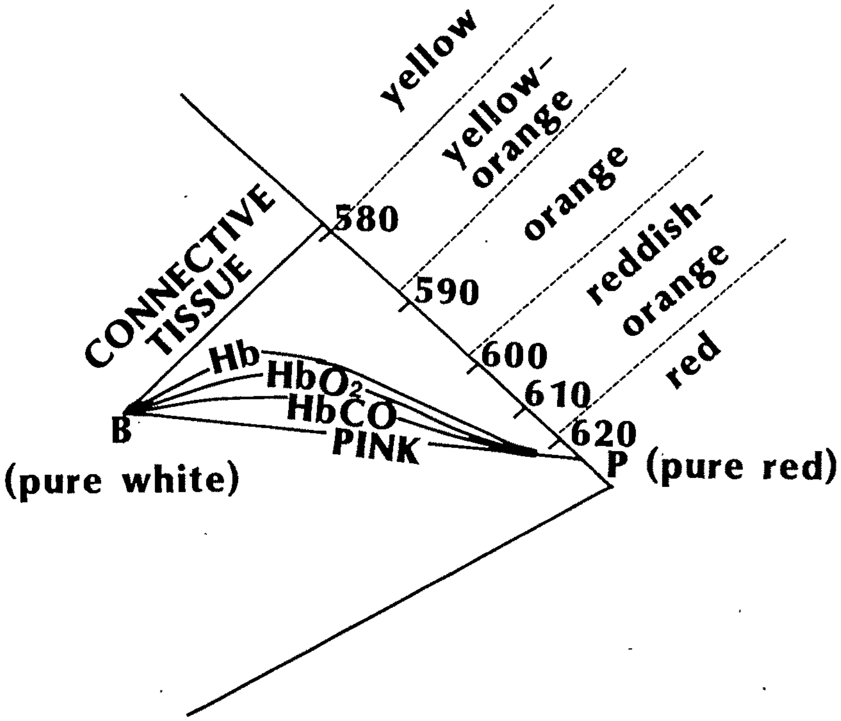

FIGURE 1. Chromaticity chart showing the change of dominant reflectance colour with changes in concentration of haemoglobin, oxyhaemoglobin and carboxyhaemoglobin. B represents the white light source, where infinitely thin films converge and lose all colour. P is the point where infinitely thick films appear as pure red. The curved lines represent the loci of colours for intermediate film thicknesses. If a straight line is drawn through B and a point representing the film thickness on the colour locus, a projection onto the wavelength scale will give the dominant wavelength seen. HbCO keeps closest to the line BP (white, to pink, to red) while Hb and HbO_2 curve upward to sweep through the yellow. Collagen reflects from a line marked 'connective tissue'.

TABLE 2. Dominant reflectance wavelengths of different connective tissues

Connective tissue	Dominant reflectance wavelength nm
Collagen powder (dry)	577
Ligamentum nuchae (washed, blood-free)	579
Ligamentum nuchae (sheep, unwashed)	580*
Ligamentum nuchae (Jersey cow)	581*
Aorta (human, young adult)	581*
Dura (human infant)	583*
Pigskin (surface)	583*
Aorta (human; post-mortem, with haemoglobin staining)	612

* On the graphs these specimens all showed the notches characteristic of HbO_2, despite the fact that no blood was visible.

Cases of carbon monoxide poisoning

Normal skin colour. This was seen in two fatal cases who survived for 3 days following accidental gassing. Normal coloured skin was also seen, among pink zones, under areas of external pressure (e.g. garments).

Grey-purple-blue skin colour. Areas of post-mortem lividity, without any pink tinge, in four cases suggested cyanosis. The skin reflectance curves mostly showed depression in the areas of haemoglobin absorption, but no clear notches. This effect was evidently the result of dark congestion of large masses of blood in the deeper venules, seen histologically to be stuffed with red cells (Table 3).

Skin colour livid, with lighter and brighter skin colour. In this group the reflectance curves were more characteristic, and histologically the subpapillary plexuses were congested. The colour could be called cherry-red (Table 3).

Intense grey-blue skin colour in patient with meprobamate poisoning. After removal from exhaust fumes this victim succumbed principally to meprobamate poisoning, after elimination of much blood CO (Table 3). We included this case because of its relevance to mixed poisonings in cases of suicide and murder.

Viewing in daylight versus sunlight. In all specimens, daylight gave a dominant wavelength about 3–4 nm lower (more yellow) than sunlight. Sunlight also tended to increase the brightness and purity of the colours.

Exhaust gas spectra. Blood from the superior sagittal sinus in the case with 80% CO-saturation (grey-blue skin colour), showed an unexpected increase in redness with dilution on filter paper. Other components in exhaust gas may perhaps exaggerate the cherry-red colour on dilution in contrast to what was found with pure CO bubbled through normal blood. Thick

TABLE 3. Optical features of skin from cases of carbon monoxide poisoning

Skin colour	Graph characteristics	Dominant wavelength nm	B %	P %	Blood CO %	Notes
Grey-blue	Uncharacteristic	600	22	28	80	See text
	Uncharacteristic	605	22	17	20	Deep skin congestion
	Suggested metHb	604	20	19	58	CO-graph on tissue solution scrape (transmission)
	Perfect HbCO-curves	599	21	22	74	Deep venous congestion
Livid–cherry-red	HbCO only	617	27	14	35	Cherry red. Superficial plexus dilated
	HbCO good fit	618	24	14	70	Colour red. Bath immersion
	Perfect HbCO	629	27	12	84	Surface vessels full. Cyanosis
Intense grey-blue	HbO_2 with metHb notch at 610 nm	611	20	14	8	Meprobamate found

smears here gave an increased dominant wavelength on dilution (608 to 626 nm, with a brightness increase from 14 to 26%).

Histology of blood vessels. In all instances where the colour was cyanotic, the dominant wavelength lay in the reddish-orange and the brightness was low. In these instances the veins in the mid- and lower dermis were widely dilated and stuffed with red cells. In some, the cells had become agglutinated and could not be displaced by stroking the surface. Arterioles, capillaries and the narrower plexuses were closed. Where the colour was pink, a large number of papillary layer vessels were open, the cross-section of the masses being much smaller. The numbers of channels packed with cells was also greater.

Low blood levels of CO. We have also seen cases with a history of gassing, but with non-fatal levels of HbCO. With survival in an uncontaminated atmosphere, the blood level falls swiftly from 80 % to 20% (a non-toxic value) in 4 h.[6]

DISCUSSION

Through earlier studies on blue skin[7] and on hair colour,[8] it became apparent that the colour produced from melanin varied with the amount and position of the pigment. A carrier medium with its own optical properties could bring about striking colour differences. Extending the study to various derivatives of haemoglobin, similar principles, and some new ones, were found to apply. It is commonly supposed that differing colours indicate specifically different substances, but this need by no means apply.

The present study has shown that an appearance of cyanosis is produced when there is deep venous filling in the mid- and lower dermis even though the blood contains oxyhaemoglobin or carboxyhaemoglobin only. The spectra are of low brightness, making the blood appear grey, with a more saturated hue, added to the bluish tint of any deeply placed absorber. To judge the colour as representing reduced haemoglobin would, therefore, be an error.

Bright red colours are seen with congestion in the superficial vascular plexus. In the present study the principal cause was the intravascular presence of carboxyhaemoglobin, in one case exaggerated by immersion in a bath where the colour was brighter and redder.

During a post-mortem examination one inevitably notices and judges the colour of the blood in a thin, often extravascular film, with a whiter background of connective tissue, fat or some white surface. It is on deliberate dilution in a clear watery medium that the dichroic behaviour becomes evident. We suggest that some of the yellowness in connective tissues is due more to the unrecognized presence of blood rather than the specific colour of elastic fibres. The colour differences between HbO_2 and HbCO are very much a product of dilution. Unless the superficial plexuses and the connective tissue reflectance combine to produce the appropriate dilution effect, the special cherry-red colour will not show in the skin.

It seems possible that necrobiosis in eccrine sweat acini starts early, but that the epidermal basal cells notably at the papillary apices suffer the same change only after temporary pressure anoxia and reactive hyperaemia. These signs have been well reviewed.[9]

If a simple test for significant CO-poisoning is needed during life, the aqueous dilution of a blood sample and comparison with a normal control may be enough to show the pink colour, and emphasise the need for prompt oxygen administration.

The 'blue' blood of reduced haemoglobin is still optically red when seen on its own, and differs more through its dark appearance (low brightness) than an actual colour difference. This paradox is the subject of a further study.

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Intralesional therapy of cutaneous leishmaniasis with sodium stibogluconate antimony

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SUMMARY

One hundred and thirty lesions of cutaneous leishmaniasis in 60 patients were treated with intralesional injections of Pentostam and 30 lesions were left untreated as controls. The injections were given at 8-day intervals and the patients followed-up for 42 days. One hundred and four lesions (80%) needed one injection only, 20 (15·4%) needed two and six (4·6%) needed three injections.

One hundred and twenty three of the treated lesions (94·6%) showed a good clinical response with complete healing or marked improvement within the follow-up period. None of the control lesions showed marked improvement or complete healing. Scarring was minimal or absent following healing of treated lesions. The only side-effect was some localized pain following the injection.

We recommend intralesional Pentostam as a safe and effective method of treating acute cutaneous leishmaniasis.

Cutaneous leishmaniasis (Baghdad boil or oriental sore) is an endemic disease in Iraq[1-3] and is now reaching epidemic proportions. It is a self-limiting disease, but usually takes several months before complete resolution is achieved and leaves disfiguring scars. Many modes of therapy have been used in this disease. Pentostam® (sodium stibogluconate) is reported to be an effective drug when given intramuscularly.[4-6] However, previous studies have questioned its effectiveness when given intralesionaly.[4,7] In the present study we have investigated the effectiveness of intralesional Pentostam® in 60 patients with cutaneous leishmaniasis.

METHODS

Sixty patients, mean age 20 years (range 5–50 years), with typical acute cutaneous leishmaniasis

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were recruited into the study. Diagnosis was confirmed in every patient clinically, as well as by laboratory demonstration of the parasite in the lesions by direct smears or cultures or both. Briefly, the lesions were cleaned with 70% ethanol and material was aspirated through a small incision made at the margin of the lesion with a sterile surgical blade. The material was stained with Giemsa and samples cultured on Noy-MacNeal Nicolle blood agar medium for up to 3 weeks to detect the presence of leishmanial promastigotes. Cultures which failed to show growth within 3 weeks were considered negative and these lesions were excluded from the study.

This examination for the protozoan was performed on all lesions before treatment, and 3 and 6 weeks after the end of treatment. Untreated control lesions were similarly examined.

Most patients had multiple skin lesions. The total number of the lesions examined was 160, of the typical wet (47) and dry (113) types. The duration of the skin lesions ranged from 1–3 months with a mean of 1·5 months. Thirty lesions (20 wet, 10 dry) in patients receiving treatment were left as controls without any treatment. One hundred and thirty lesions (90 wet, 40 dry) were treated with Pentostam®. These lesions were injected every 8 days and followed up for 42 days after the last injection. The number of injections needed depended on the response of the particular lesion to the treatment. Sixty one lesions (41 dry, 20 wet) needed one injection and 66 (50 dry, 16 wet) needed two injections. The remaining three lesions (two dry, one wet) needed three injections.

Pentostam® 100 mg/ml was diluted 1 in 3 with 1% xylocaine (1 ml 100 mg/ml Pentostam plus 2 ml 1% xylocaine). The amount required was 2–4 ml depending on the size of the lesion. The solution was infiltrated into the lesion from all sides until the whole lesion had blanched.

The response to treatment was graded as: slight, decreased erythema and oedema of the lesion; moderate, reduction in the size of the lesion by up to 30%; marked, reduction in the size of the lesion by > 60%, and parasites not detected in the lesion by smears or culture; and total clearance of the lesion with parasites not detected in the affected site by smears or culture.

RESULTS

The lesions treated with Pentostam® showed improvement usually within 7–10 days after the

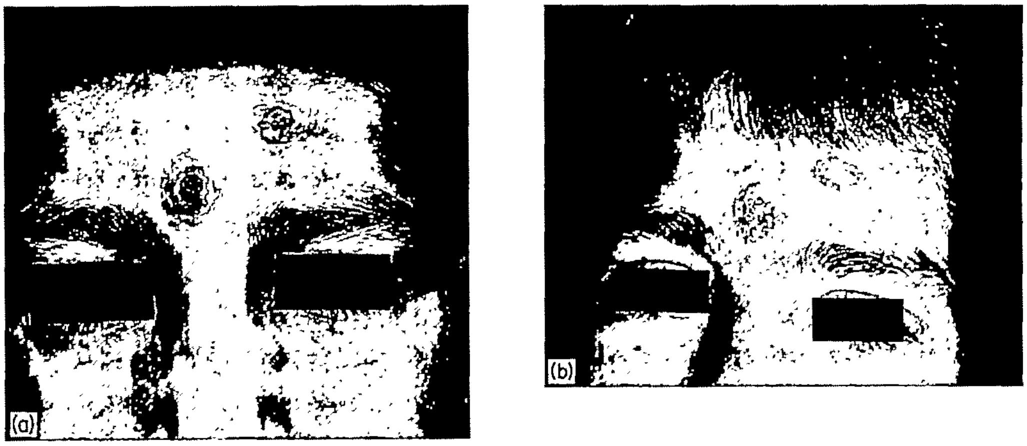

FIGURE 1. (a) Wet crusted lesions of cutaneous leishmaniasis on the forehead of a 13 year-old boy, which had been present for 6 weeks. (b) Appearance of lesions 3 weeks after treatment with a single injection of Pentostam.

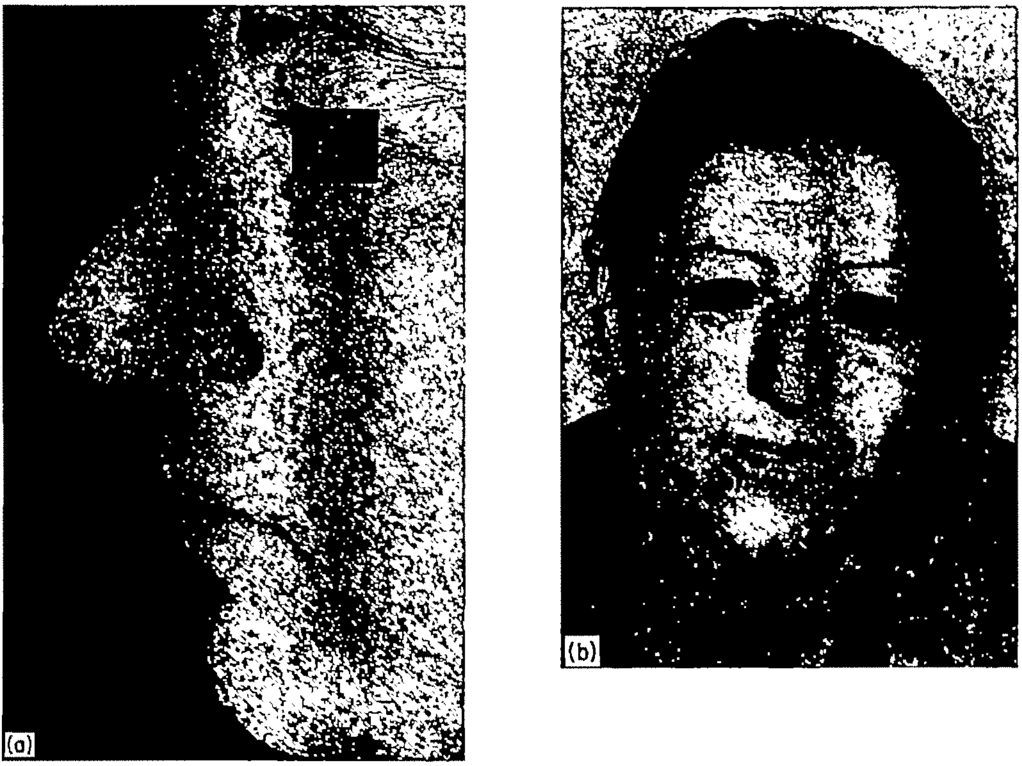

FIGURE 2. (a) Disfiguring crusted lesion of cutaneous leishmaniasis on the nose of a 45 year-old woman, which had developed over a period of 7 weeks. (b) Appearance 5 weeks after two injections of Pentostam, spaced 8 days apart.

first injection. One hundred and twenty three lesions (94·6%) showed either marked improvement or total clearance within 42 days follow-up and no parasites were detectable in these lesions. Examples are shown in Figures 1 and 2.

The remaining seven lesions (5·3%) showed slight to moderate improvement. The time to resolution ranged from 2–6 weeks with a mean of 4 weeks. The number of injections needed was usually one or two, rarely were further injections needed. There were no side-effects following the treatments apart from some pain initially at the injection site. After healing scarring was minimal or absent, but hyperpigmentation and scaling was noted in 50 patients.

The 30 control lesions showed no sign of healing after 42 days follow-up except for four lesions which showed slight clinical improvement although parasites were still detectable in these lesions by smears or culture.

DISCUSSION

Although cutaneous leishmaniasis is a self-limiting disease, it is disfiguring. It may persist for several months, leaving ugly scars. The aim of therapy is to shorten the duration of the lesions and to prevent scarring. The ideal treatment should be as simple as possible and should have no side-effects.

There are many nodes of therapy of variable effectiveness used to treat cutaneous leishmaniasis. Local treatment such as intralesional injection of berberine chloride,[8] berberine disodium citrate,[9] berberine sulphate, mepacrine and emetin,[3,5] as well as cryotherapy[10] and infra-red therapy,[3] have been tried. Topical therapy with poromomycin has recently been described to be very effective.[11] Systemic drugs have also been used including stibophen (Fuadin),[12] rifampicin,[6,13] metronidazole,[14–16] and cotrimoxazole,[17,18] with variable results and more recently levamisole has proved successful in chronic cutaneous leishmaniasis.[19] Studies have suggested that the new antifungal agent ketaconazole may be an effective therapy for both cutaneous and mucocutaneous leishmaniasis.[20,21] However, Pentostam is still generally regarded as the single most effective systemic drug.[4–6,12] It is given intramuscularly or intravenously at an adult dose of 600 mg daily on at least 10 successive days and further courses may be needed. Its mode of action is unclear, but it has been shown to selectively inhibit a number of enzymes of the parasite, most importantly, phosphofructokinase which catalyses a rate limiting step of glycolysis.[22] Paranteral Pentostam, however, has been associated with side-effects including nausea, vomiting, skin rashes, elevated liver enzymes, nephropathy, cardiac arrhythmias and non-specific T changes on ECG. Intralesional therapy with Pentostam is not widely used and there is some controversy about its effectiveness.[4,7,12] More recently, however, the W.H.O.[5] have reported that the intralesional treatment of cutaneous leishmaniasis is effective and recommend its use. The present study supports these recommendations and has shown that intralesional Pentostam is very simple to use and is clinically effective, requiring only one to two injections at 8-day intervals.

The technique of intralesional injection is very important to the outcome of treatment. The injections should be given all around the advancing edge of the lesion, and infiltration should continue until the whole lesion has blanched. We believe that inadequate infiltration of lesions is probably the reason for the reported failure of this treatment. Local injection of Pentostam may be painful, but this can be overcome by addition of 1% xylocain to the Pentostam before injection without reducing its effectiveness.

The present study has shown that intralesional Pentostam, when used correctly, is a safe and effective method of treating acute cutaneous leishmaniasis and we advocate its general use in this condition.

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Photodynamic therapy: a better treatment for widespread Bowen's disease

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SUMMARY

We report here the use of photodynamic therapy to treat two patients with multiple lesions of Bowen's disease. A total of over 500 lesions were treated, less than 10% requiring two treatments, and at follow-up 6 months later no lesions remained in either patient. The only important side-effect of treatment was a marked photosensitivity reaction. We consider photodynamic therapy an efficient treatment for Bowen's disease; multiple lesions can be treated in a short treatment session, without local anaesthesia, and healing occurs within 2 weeks.

Photodynamic therapy is a new method of tumour destruction which is currently being extensively researched, as a treatment modality for widespread, superficial malignant disease.

Photodynamic therapy is administered by first giving the subject a slow intravenous injection of a photosensitizing agent. After injection the photosensitizing agent becomes widely distributed throughout the body, but is selectively retained by malignant tissues giving, after 72 h, a higher level of the sensitizer in the tumour than in the surrounding normal tissues. The photosensitizing agent is then activated by light of a wavelength specific to it. This photoactivation causes the release of a toxic substance or substances causing tumour destruction, with minimal damage to surrounding normal tissues which contain lower levels of the sensitizer.

The first such agent to be described was haematoporphyrin derivative (HPD). Lipson *et al.*[1] showed that after systemic administration, HPD caused areas of fluorescence in the presence of UV light which correlated closely with the distribution of malignant tissue. When HPD is photoactivated by red light, singlet oxygen is produced by energy transfer from the excited porphyrin molecule.[2] It was shown that these highly energized oxygen radicals oxidize sensitive bonds and are, therefore, cytotoxic to the sensitized tissue causing lysis of most tumour cells and congestion of the larger tumour vessels within one hour of light treatment. The active component has since been identified as di-haematoporphyrin ether (DHE) and this agent is now available as a photosensitizing agent.

Bugelski *et al.*[3] have shown that HPD is at first widely distributed throughout the body and

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then, whilst normal parenchymal tissues clear HPD as serum levels fall, malignant tissue exhibits predominant accumulation of HPD in the stroma. This is attributed to the increased vascular permeability of tumours and to poor lymphatic clearance. Both HPD and DHE are retained by some normal tissues which include liver, spleen, kidneys and skin and can induce sunburn reactions after normal exposure to sunlight for up to 1 month.

We report here two cases of Bowen's disease treated with photodynamic therapy.

METHODS

Treatment protocol

The treatment was carried out on an out-patient basis. On day 1 the patients received a slow intravenous injection of DHE (1·5–2 mg/kg body weight). No significant side effects have been reported from the injection. The patients were then warned to avoid sunlight for 4 weeks.

After 72 h the patients returned for photo-irradiation using a laser light source. The laser is ideal for this providing a high energy, pure light source of constant wavelength. The wavelength required for photoactivation of HPD (and DHE) is 630 nm which also penetrates tissues to a depth of up to 5 mm. This light may be produced either by a tuneable dye laser or, as in our department, by a pulsed gold vapour laser (provided by Oxford Lasers in a short collaborative study) which produces high levels of power at a wavelength of 628 nm. The high power levels available with the gold vapour laser allows larger tumours to be treated in a shorter time. The total dose was 25 J/cm^2. The treatment was carried out without local anaesthetic and rapid destruction of multiple lesions could be completed in a half hour treatment session.

After treatment, lesions became erythematous and later scabbed over, with complete healing by 14 days.

CASE REPORTS

Case 1

A 69-year-old lady presented 30 years after a 5-year course of arsenic, taken for treatment of psoriasis, with 15 basal cell carcinomas and 35 widespread lesions of Bowen's disease confirmed histologically. The lesions were considered too widespread for conventional therapy so she was referred for photodynamic therapy.

Treatment was carried out by photosensitization with an intravenous injection of DHE (2 mg/kg) and 72 h later all 50 lesions were treated with red light (628 nm), at doses of 25 J/cm^2 to the Bowen's lesions and 50 J/cm^2 to the basal cell carcinomas. Treatment was completed within an hour. Mild erythema was present at 1 week, but healing was complete by 14 days. Six months following treatment only one lesion remained and there had been no recurrences.

There was a marked photosensitivity reaction following treatment.

Case 2

A 49-year-old lady was referred for photodynamic therapy of 52 widespread lesions of histologically confirmed Bowen's disease. She had previously had over 60 lesions excised and further unsuccessful treatment with two courses of 5-fluorouracil.

Treatment was again carried out using DHE (2 mg/kg) and red light as in Case 1. All 52 lesions were treated within an hour. The lesions healed in 10 days, and 4 months following treatment only five of the original lesions remained. However, a crop of new lesions had appeared, and at a second session of photodynamic therapy, 84 lesions were treated. Six months following this second treatment only six out of a total of 131 treated lesions remained. After each treatment there was a marked photosensitivity reaction.

Further new lesions developed which were treated at a third session using half the dose of DHE (1 mg/kg) and twice the light dose in an attempt to reduce skin photosensitization. However, the reaction was as severe as before and only half of the 90 treated lesions responded to treatment.

Two more treatment sessions using the original treatment protocol were carried out to treat these remaining lesions and further new lesions. A total of more than 500 lesions were treated altogether, and 6 months following the last treatment no lesions remained and no new lesions had developed.

DISCUSSION

Single lesions of Bowen's disease can be satisfactorily treated by surgical excision, curettage, cautery, cryotherapy or topical application of 5-fluorouracil.[4,5] None of these methods is an ideal treatment for a patient with widespread Bowen's disease because many treatment sessions are required and the treated areas take between 2 and 4 weeks to heal depending on the modality chosen. Where photodynamic therapy facilities are available, this mode of treatment offers the twin advantages of speed of treatment and rapid healing. However, increased photosensitivity can be a problem for up to a month. Halving the dose of the photosensitizing agent (DHE) did not reduce the skin reaction, but significantly reduced the efficacy of the treatment. Treatment of non-urgent conditions like Bowen's disease is, therefore, best delayed until the winter months.

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Erythropoietic protoporphyria exacerbated by oral iron therapy

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SUMMARY

Four patients with erythropoietic protoporphyria (EPP) are reported, in whom oral iron produced clear-cut clinical and biochemical deterioration. This suggests that there are two biochemically and genetically distinct sub-groups of EPP patients distinguished by their dramatically different response to oral iron.

Erythropoietic protoporphyria (EPP) is thought to be due to an inherited defect in the terminal step of the haem biosynthetic pathway in which protoporphyrin IX and iron combine to form haem.[1] The result of this is an elevated level of protoporphyrin IX in the tissues. The diagnosis is confirmed by the presence of elevated levels of this haem precursor in red blood cells and stools.

In iron deficiency, the protoporphyrin IX level is also elevated, but this returns to normal when iron is administered. Iron stores have been found to be low in some patients with EPP,[2] and iron therapy has been found to be of benefit in some cases.[3] We report here four patients, who showed clear evidence of deterioration following oral iron.

CASE REPORTS

Case 1 (Previously reported by Baker[4])

This female patient, born in 1945, had suffered episodes of redness, swelling and tenderness of the skin on exposure to sunlight from childhood until the age of 18 years. There were no further episodes, despite summer holidays by the Mediterranean, until March 1970 when she was found to have an iron deficiency anaemia and was started on oral iron. Two months later she developed severe photosensitivity.

Investigations at that time showed a normoblastic marrow with low iron stores and a red cell protoporphyrin level of 18,432 nmol/l (normal 70–925 nmol/l). A diagnosis of EPP was made. It

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then transpired that a grandmother and a brother were intolerant of sunlight and both were found to have biochemical evidence of EPP.

Iron therapy was withdrawn and the patient's photosensitivity subsided over the following 6 to 8 weeks. There was no adverse reaction to further sun exposure. The red cell protoporphyrin level also fell when iron was withdrawn (Table 1).

However, as her haemoglobin level began to fall, further oral iron was started in September 1970. Two weeks later, the protoporphyrin level began to rise and within 6 weeks of restarting iron she developed tenderness of the dorsa of both hands, but there was no erythema. Iron was, therefore, stopped again.

Case 2 (Previously reported by Graham-Brown et al.[5]*)*

This female patient, born in 1972, was diagnosed at the age of 18 months as suffering from the eosinophilic granuloma form of histiocytosis X. There were associated changes in her liver. From the age of 2 years she had suffered from burning and redness of skin exposed to sunlight and in August 1982 she was admitted to hospital with a severe photosensitivity reaction. At this time, she was on oral iron because of anaemia resulting from bleeding from oesophageal varices. A skin biopsy from the dorsum of the hand showed large deposits of PAS positive material around the upper dermal vessels—a change compatible with protoporphyria. A porphyrin screen showed a raised red cell protoporphyrin level of 4,077 nmol/l (normal 70–925 nmol/l). Her serum iron level was raised and, therefore, iron therapy was stopped. Her symptoms improved rapidly and her protoporphyrin level and serum iron levels fell considerably (Table 1).

Because the association between her sun sensitivity and the iron treatment was not appreciated at that time, she was restarted on 200 mg of ferrous sulphate daily by mouth. Within a few days she complained of burning of skin exposed to moderate sunlight.

TABLE 1. Levels of red cell protoporphyrin, serum iron and serum ferritin in our four patients on and off oral iron therapy

			Red cell protoporphyrin nmol/l (normal range 70–925)	Serum iron μmol/l (normal range 11–36)	Serum ferritin μg/l (normal range 10–150)
Case 1	May 1970	On iron	18,432	ND	ND
	July 1970	Off iron	5,652	ND	ND
	November 1970	On iron	17,154	ND	ND
Case 2	August 1982	On iron	4,077	40	ND
	January 1983	Off iron	945	20	ND
Case 3	January 1984	Off iron	2,571	ND	4
	February 1984	On iron	8,724	ND	5
	April 1984	Off iron	5,139	ND	4
Case 4	January 1984	Off iron	4,579	ND	2
	March 1984	On iron	7,674	ND	10

ND = not determined.

Case 3

This female patient, born in 1967, was diagnosed as having EPP in 1970. She was further investigated in 1984, when a biopsy from the dorsum of the hand showed PAS positive material around dermal vessels. Her red cell protoporphyrin level was raised at 2,571 nmol/l. A blood screen showed hypochromia and microcytosis, but a normal haemoglobin level. The serum ferritin level was low at 4 μg/l.

In view of her low iron level, she was started on oral ferrous sulphate. One month later, she developed severe burning of the skin in weak British sunlight. Her red cell protoporphyrin level had risen (Table 1). When iron therapy was stopped, there was a subsequent improvement in symptoms and the protoporphyrin level fell to near normal.

Case 4

This patient, the sister of Case 3, was born in 1964, and was also investigated in 1984. A skin biopsy from the dorsum of her hand showed PAS positive material around dermal vessls, and the red cell protoporphyrin level was elevated at 4,579 nmol/l, confirming the diagnosis of EPP. She was iron deficient, and following oral ferrous sulphate, showed symptomatic and biochemical deterioration similar to that noted in her sister.

In Cases 3 and 4, fibroblast ferrochelatase levels were assayed and were found to be low in both patients. Their mother, father and two sisters had no demonstrable biochemical abnormality.

DISCUSSION

Erythropoietic protoporphyria was first described by Magnus *et al.*[6] in a patient with photosensitivity and increased protoporphyrin levels in the stool and red blood cells. The mechanism of the disease was unclear, but it is now thought to be due to a defect in the chelation of iron to protoporphyrin to form haem. This reaction is catalysed by the enzyme ferrochelatase or haem synthetase[7] which has been assayed in a variety of tissues including liver, erythrocytes, bone marrow and fibroblasts.[7,8] Levels of ferrochelatase are low in EPP, suggesting that lack of the enzyme may be responsible for the disease. Family studies have suggested either that EPP is inherited on a single gene as an autosomal dominant trait[9,10] or that a second autosomal recessive gene is required.[11] The condition usually runs a benign course with photosensitivity being the most troublesome symptom.

There is no effective treatment, although a number of treatments have been tried for relief of the photosensitivity, including oral beta-carotene.[12,13] However, there have been deaths due to hepatic failure in EPP.[14,15] Low iron stores have been found in some patients and oral iron has been tried with some success. It has been suggested that iron may enhance remaining enzymatic activity or induce a non-enzymatic pathway of haem production.[3] Improvement has occurred in patients following infusions of haematin. This is thought to help because haematin inhibits delta aminolevulinic acid synthetase, the initial enzyme in the porphyrin pathway.[16] Blood transfusion has also been reported to be effective, its probable mode of action being the suppression of erythropoietin.[17]

In our four patients the reverse of this appears to have occurred. All four were anaemic or had low iron stores or both and were, therefore, started on oral iron. There was striking symptomatic and biochemical deterioration within a few weeks of starting treatment in all four patients. Improvement followed when iron was withdrawn. In Case 2 there was coexistent histiocytosis X with liver involvement, which may have contributed to the deterioration.[18] However, the periods of exacerbation of the photosensitivity seemed to coincide with the times when she was on iron therapy.

It seems very unlikely that the response to iron therapy would be so variable if a single enzymatic defect were responsible for all cases of EPP. In view of the fact that genetic heterogeneity in EPP has been suggested previously,[11] we consider that the dramatic and unexpected deterioration of the disease in our patients following oral iron provides further evidence that there are at least two different sub-groups of patients with EPP, who appear to be biochemically, and are probably also genetically, distinct.

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Treatment of chronic urticaria with cetirizine dihydrochloride a non-sedating antihistamine

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SUMMARY

The efficacy of cetirizine dihydrochloride, a new H_1-antagonist with minimal sedative or anticholinergic side effects was evaluated in 30 patients with chronic idiopathic urticaria. In the first part of the study, cetirizine 10 mg and placebo were compared in a double-blind cross-over trial. In the second part, patients who did not respond adequately in the first part were randomized, still double-blind, to receive 10 mg cetirizine either once daily or twice daily.

In the first part, treatment was discontinued by 17 patients on placebo and two patients on cetirizine because of lack of efficacy. Cetirizine dihydrochloride was found significantly to reduce occurrence of weals, erythema and pruritus compared with placebo ($P<0{\cdot}001$). Twenty-six of the patients improved on cetirizine and two on placebo. Mild sedation was noted by two patients on cetirizine and by one on placebo.

Cetirizine dihydrochloride is a new anti-allergic compound, a carboxylic analogue of hydroxyzine. It is a potent H_1 receptor blocker,[1,2] with a minimal sedating effect as demonstrated in healthy subjects and in patients with cold uriticaria and dermographism[3] as well as in patients with asthma and allergic rhinitis.[4] A single dose of 10 mg cetirizine dihydrochloride strongly inhibits the weal and flare produced by intracutaneous injection of histamine, compound 48/80 and grass pollen extracts.[3,5] It also inhibits dermographism and the weals produced by an ice-cube test in patients with cold urticaria.[3] The effect on the skin is maximal about 4 h after ingestion and lasts for 24 h. We report here a double-blind trial of the effect of oral cetirizine dihydrochloride in 30 patients with severe chronic idiopathic urticaria.

METHODS

Patients

We studied 30 patients (11 men and 19 women) aged from 15 to 70 years (mean 43 years) suffering from chronic idiopathic urticaria. Most had suffered daily eruptions of weals for between 2 months and 20 years (mean 4 years). All patients had avoided drugs which could be responsible for their urticaria. None were taking aspirin or other anti-inflammatory drugs. They had tried to avoid benzoates, azo dyes and yeasts in their diet, but continued to have attacks of urticaria at least 3 days per week. Patients with angioedema and pressure urticaria were excluded. All patients had previously been treated with various antihistamines. The most frequently used were hydroxyzine, dexchlorpheniramine and clemastine. In 23 of the patients there had been a favourable effect, whereas seven reported no definite effect. Sedation and a dry mouth had been the main side effects. The antihistamines as well as anticholinergics and $\beta 2$ agonists were stopped for at least 4 days before the trial. None of the patients had used systemic corticosteroids during the previous 2 months.

Study design

The study consisted of two parts. Part I was a double-blind, cross-over trial comparing cetirizine 10 mg in the evening with placebo. Each treatment period lasted 15 days with no wash-out period in between. Part II was a double-blind trial with the patients divided into 2 groups, comparing placebo in the morning + 10 mg cetirizine in the evening with 10 mg cetirizine morning and evening. Patients who did not respond adequately to either treatment in Part I were enrolled into Part II.

Procedure

The patients recorded daily the occurrence weals, erythema, pruritus and angioedema, rated on a 4-point scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe). The daily results were then used to calculate a mean score for the week. The patients were seen after each 2-week treatment period, at which time the physician graded the symptoms, using the same scale. The patients were also asked to assess their condition on an unmarked 100 mm visual analogue scale, ranging from 0 = very poor to 100 = excellent, and at the end of the study to indicate the preferred treatment period.

The investigator made a global evaluation of the results of the treatment in relation to the pre-treatment symptom score rating the percentage improvement as: excellent, 91–100%; very good, 71–90%; good, 51–70%; average, 31–50; poor ⩽30%.

Safety was monitored by recording daily any adverse reactions and the results of laboratory tests carried out including full blood counts with differential counts, and biochemistry including serum creatinine, bilirubin, alkaline phosphatases, aminotransferases (SGOT, SGPT) electrolytes and triglycerides, and tests for albumin and glucose in the urine. The patients were free to stop the treatment but in this event had to inform the investigator immediately.

Statistical analysis.

In Part I, a paired-sign test was used to compare the individual symptom scores, the mean weekly symptom scores and the global evaluation of treatment on cetirizine and placebo. Visual analogue scores were compared using a Wilcoxon signed rank test. A binominal test was used to compare preferred treatment periods and the proportion of patients in each group who discontinued treatment because they found it ineffective.

RESULTS

Cetirizine dihydrochloride (10 mg) taken in the evening significantly reduced occurrence of weals, erythema and pruritus compared with placebo ($P < 0{\cdot}001$). The scores for pruritus and weals before and after treatment as judged by the investigator are shown in Table 1. The effects on erythema were almost the same as for the weals. The weekly symptom scores were also significantly lower on cetirizine than on placebo ($P < 0{\cdot}001$). The visual analogue scores are

TABLE 1. Distribution of scores for pruritus and weals before and after treatment with placebo or cetirizine dihydrochloride

Symptom	Score	No. of patients Before treatment	Placebo	Cetirizine
Pruritus	0	1	0	19
	1	2	6	6
	2	16	13	4
	3	11	11	1
	mean score	2·2	2·2	0·6
Weals	0	2	2	20
	1	1	4	6
	2	20	17	3
	3	7	7	1
	mean score	2·1	2·1	0·6

TABLE 2. Visual analogue scores* before treatment and after treatment with placebo and with cetirizine ($n = 30$)

	Mean	Median	Range
Before treatment	28·1	10·5	2–78
Cetirizine	80·8**	92·5	65–99
Placebo	24·1	15·5	6–67

* 0 = no effect; 100 = free from symptoms

** Significantly greater than placebo ($P < 0{\cdot}001$)

shown in Table 2, the global evaluations by the patients and by the investigator are shown in Tables 3 and 4, respectively and the preferred treatment periods in Table 5. The results with cetirizine were significantly better than those with placebo throughout ($P < 0{\cdot}001$).

Mild sedation was noted by two patients on cetirizine and by one patient on placebo. One patient complained of a dry mouth and one of diarrhoea while on cetirizine. One patient reported feeling a heaviness in his head on both cetirizine and placebo. None of the patients discontinued treatment because of these side effects, but 17 patients on placebo and two on cetirizine discontinued treatment after 5–8 days because of lack of efficacy. The patients were then crossed over or enrolled into Part II. The code was not broken before the study was completed.

Six patients with a poor or average response to both placebo and cetirizine continued in the second part of the study. They had previously experienced little benefit from several antihistamines. Three of them received 10 mg cetirizine, which they judged to have an average effect and three received 20 mg which one judged as excellent, one average and one poor. No side-effects were noted after 20 mg cetirizine.

The treatments did not induce any abnormal changes in the laboratory tests.

TABLE 3. Patients' global evaluation of treatment with 10 mg cetirizine or placebo

	No. of patients	
	Placebo	Cetirizine
Much improved	0	16
Improved	3	10
No change	18	3
Worse	7	1
Much worse	2	0

TABLE 4. Investigator's evaluation of treatment with 10 mg cetirizine or placebo

	No. of patients	
	Placebo	Cetirizine
Excellent	0	13
Very good	1	7
Good	2	3
Average	2	4
Poor	25	3

TABLE 5. Preferred treatment period

	Investigator	Patient
Cetirizine	25	25
Placebo	1	2
Neither	2	1
Both	2	2

DISCUSSION

When managing patients with urticaria it is important to search for factors which might provoke the eruption. Treatment with antihistamines is frequently needed for certain periods. A drawback with most antihistamines has been their sedative effect. A new generation of H_1 blockers with little or no sedative effect is being developed. Good results in chronic urticaria have been reported with terfenadine, astemizole, acrivastine and loratidine.[6–9] Cetirizine dihydrochloride has been shown to be effective in other allergic disorders, and the present study has shown it to be effective in chronic urticaria. A daily dose of 10 mg seemed to be optimal for most patients although a higher dosage could be of value in certain patients. After stopping the treatment the weals usually reappeared after 24–48 h indicating a rather short duration of action of the drug. Cetirizine dihydrochloride had no significant sedative effect, which for most patients was a great advantage. It is difficult from the present study to compare the effects of cetirizine with those of other antihistamines. The patients who responded well to cetirizine had also responded to some extent to other antihistamines and those with a poor response to cetirizine had found other antihistamines to be ineffective. There was, however, a group of patients who found cetirizine to be the only drug which kept them free from urticaria.

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Intermittent high-dose dexamethasone-cyclophosphamide therapy for pemphigus

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SUMMARY

Since 1982, we have treated 79 pemphigus patients with an arbitrarily designed regimen of 100 mg dexamethasone dissolved in 5% glucose given by an intravenous infusion over 1 h, daily on 3 consecutive days and in addition, 500 mg cyclophosphamide on day 1 only. The intermittent high doses (IHD) of dexamethasone are repeated every 2–4 weeks, and the patient continues to take 50 mg/day oral cyclophosphamide.

This treatment is divided into four phases. During Phase I, the patient continues to develop relapses of pemphigus a variable number of days after IHD, but the lesions heal up quickly after IHD. These relapses become progressively milder and stop after a few months, but the IHD are continued once a month for 6–9 months (Phase II). In the next phase (Phase III), the monthly IHD are stopped, and the patient continues to take 50 mg/day cyclophosphamide orally. After approximately 1 year this maintenance treatment is withdrawn and the patient is observed for any relapses (Phase IV).

Of the 79 patients treated, 10 patients have been lost to follow-up and two have died, one due to leukopaenia caused by inadvertent additional administration of methotrexate, and the other of an unknown cause. Of the remaining 67 patients, 25 are off treatment (Phase IV), 25 are taking only 50 mg cyclophosphamide daily (Phase III), ten are also in remission, but still receiving intermittent high doses of dexamethasone-cyclophosphamide (Phase II), and seven still have active disease (Phase I). The remissions are long lasting, being already more than 2 years in 17 patients (maximum 46 months), 1–2 years in 11 and less than 1 year in 32.

Side-effects of corticosteroids and cyclophosphamide are minimal and not clinically significant except for increased susceptibility to infections.

A variety of drugs and therapeutic regimens have been used for the treatment of pemphigus, but pemphigus continues to be a potentially fatal disease,[1] death being due either to the disease or to the complications of treatment.[2–6] Our personal past experience with pemphigus has been similar.[7] Early intensive treatment can, however, significantly reduce the mortality and morbidity of this disease.[8]

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In 1982, we started using an arbitrarily designed combination of high dose dexamethasone and cyclophosphamide intermittently, and low dose cyclophosphamide continuously. Subsequently, we reported[9] the two chief advantages of this regimen, which are rapid healing of the pemphigus lesions, so that in-patient therapy was reduced to only 4–5 days at a time, and freedom from such side-effects as obesity, osteoporosis, hypertension, diabetes, and peptic ulceration caused by prolonged corticosteroid therapy.

Two years ago, we observed that a proportion of our patients treated earlier with this regimen, were in remission even after they stopped receiving treatment, and the disease-free remissions in some cases were 2 years or longer.[10] We have subsequently treated more patients with this regimen, the results of which we now report.

METHODS

The diagnosis of pemphigus was made on clinical criteria, Tzanck smears and histopathology. Immunofluorescence studies were not undertaken due to lack of facilities. Laboratory evaluation included haemoglobin, total and differential leukocyte counts, platelet counts, ESR, blood glucose, blood urea, SGOT, SGPT, serum alkaline phosphatase, serum electrolytes, urinalysis, stool examination for occult blood and chest X-ray. All were undertaken before starting treatment and during follow-up. In addition, periodic examination for cataracts, stria atrophicans, blood pressure, history of epigastric pain, weight change, hair loss and urinary symptoms were carried out to record side-effects of corticosteroids and cyclophosphamide.

The treatment regimen used consisted of two components. These were intermittent high dose (IHD) therapy consisting of 100 mg dexamethasone in 5% glucose given by a slow intravenous infusion over a period of 1–2 h on 3 consecutive days, together with 500 mg cyclophosphamide given in the same intravenous drip on the 1st day only, and continuous low dose (CLD) therapy consisting of one tablet of 50 mg cyclophosphamide daily orally between the courses of intermittent high dose therapy.

The entire treatment was divided into four phases. During the first phase, pemphigus lesions would heal rapidly following the IHD, but after a variable number of days, new lesions would appear. IHD was repeated every 2–4 weeks depending upon the state of clinical activity, while CLD was continued irrespective of the results. After a variable number of courses of IHD, the relapses would start becoming milder and ultimately the patient would go into the next phase, the phase of remission while on therapy. During this phase (Phase II), the courses of IHD were repeated every month for a period of at least 6 months, and CLD continued. After the patient had remained in remission for a minimum of 6 months, the courses of IHD were stopped, but CLD was continued (Phase III). After 1 further year of Phase III, CLD was withdrawn, and the patient was followed up without any treatment (Phase IV).

RESULTS

Between January 1982 and May 1987, 79 patients (74 pemphigus vulgaris, three pemphigus foliaceus, and two pemphigus erythematosus) aged between 10 and 60 years, were treated with this regimen. Of these, 10 patients have been lost to follow-up and two have died. Of the remaining 67 patients, 60 patients are in remission at the time of writing, while seven are relapsing between IHD courses. In 25 patients all treatment has been withdrawn (Phase IV), and 25 patients are receiving only a maintenance dose of cyclophosphamide (Phase III). In 10 patients, the remission is recent and these patients are still in Phase II (Table 1). At the time of

The purpose of the present report is to encourage other centres to initiate a wide evaluation of our regimen, and to initiate trials of further modifications of this regimen to establish optimal dosage schedules.

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Effect of isotretinoin on eccrine gland function

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SUMMARY

Sweat secretion rate, stimulated by iontophoresis of pilocarpine, was measured before and during treatment with isotretinoin in 20 patients. Sweat secretion was increased during treatment in 15 patients (75%), but the mean increase of 15% was not statistically significant ($P=0{\cdot}085$, paired *t*-test; 95% confidence limits $-0{\cdot}2\%$ to $+30\%$) and no patients had a clinically apparent alteration of sweating. We conclude that there is no important change in stimulated sweating associated with isotretinoin therapy.

Isotretinoin (13-*cis* retinoic acid) has a major modulatory effect on sebaceous gland function, sebum excretion being decreased by over 85% after 4 weeks of treatment.[1] Effects of retinoids on apocrine and eccrine glands are less certain. Because of the putative relationship between apocrine gland dysfunction and hidradenitis suppurativa, isotretinoin has been used in the therapy of this condition,[2,3] but results have not been impressive. Even less is known about possible effects of retinoids on eccrine function, although some studies have reported subjective abnormalities of sweating in patients treated with oral retinoids.[4–6] Cholinergic stimulation of sweating using pilocarpine iontophoresis provides a reliable test of sweat gland function and has been used to measure sex and age differences in sweating.[7] We have used this method to study the effect of isotretinoin on eccrine gland function.

METHODS

The stimulated sweat secretion rate was measured in 15 male and five female patients aged between 14 and 40 years who were attending our Dermatology out-patient Department for treatment of acne. All patients were considered suitable for treatment with isotretinoin, having had poor therapeutic responses to previous treatments with topical agents and adequate courses of oral antibiotics. No patient had previously received oestrogens or anti-androgens. The dosage of isotretinoin was 0·5–1·0 mg/kg/day, as determined by the clinician managing the individual patients.

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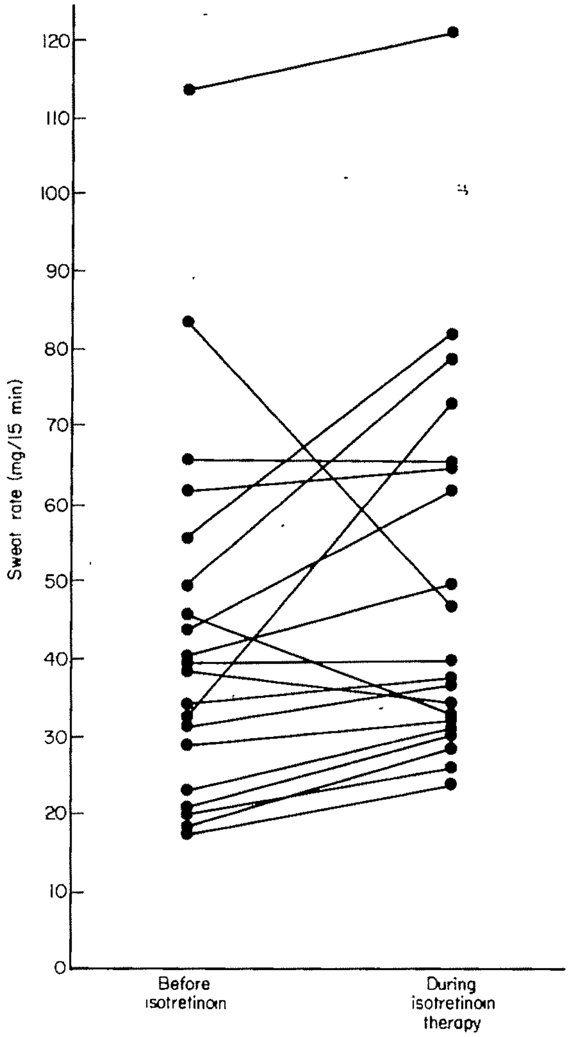

FIGURE 1. Effect of isotretinoin on stimulated sweating in 20 patients.

Sweating was measured immediately before starting treatment and on a single occasion between the 4th and 8th week of treatment; patients were also asked if they had noted any subjective change in sweating.

Sweating was induced on the flexor side of the forearm by iontophoresis of 0·2% pilocarpine chloride with a current density of 4 mA for 5 min.[7] The skin was then dried carefully and an aluminium capsule stuck to the skin with adhesive. Sweat was collected on 3 cm circles of filter paper placed inside the capsule for 15 min, which were weighed immediately after collection. Sweat output was expressed as mg/15 min. Results before and during treatment were compared using Student's paired *t*-test.

RESULTS

Sweat rates before and during therapy with isotretinoin are shown in Figure 1. In 15 of the 20 patients studied there was an increase in sweat rate, in one there was no change and in four there was a decrease. None of the patients noted any subjective change in sweating. The mean sweat rate (± SEM) before isotretinoin therapy was 43·4 ± 5·4 mg/15 min compared with 49·9 ± 5·6 mg/15 min during treatment, a mean increase of 6·5 mg/15 min (15%). This change was not statistically significant and the 95% confidence limits for the estimate were −0·7 mg/15 min to +13·6 mg/15 min (−2% to +30%).

DISCUSSION

Oral retinoid drugs have diverse effects on skin and some studies of clinical use in various dermatoses have reported subjective alteration in sweating as a possible side-effect. In our study there was a slight increase in pilocarpine-stimulated sweating in most patients during treatment with isotretinoin. However, the increase was not statistically significant and the 95% confidence limits for the mean change included zero. Although the test we used measures stimulated eccrine function, and may not necessarily reflect basal sweat rate, the results suggest that any effect of isotretinoin on eccrine function is small and clinically unimportant. No patient noted any subjective alteration in sweating.

It is of interest that most reports of increased sweating as a side-effect of retinoids have been made during studies using etretinate rather than isotretinoin.[4–6] However, one study of etretinate or placebo combined with photochemotherapy recorded an equal number of patients with increased sweating in the etretinate and placebo groups.[8] By contrast, one patient treated with isotretinoin by Lester *et al.*[9] was reported to have decreased sweating as a side-effect. Although side-effects of etretinate and isotretinoin have many similarities, there are quantitative differences between them[10] and it is possible that etretinate has a greater effect on sweating than isotretinoin. However, preliminary studies of stimulated sweating in patients taking etretinate do not suggest that this drug has a major effect on sweat rates (Rees and Cox, unpublished observations).

All our patients experienced the anticipated cutaneous side-effects of isotretinoin and there was an improvement in their acne. The fact that there was little effect on sweat gland function suggests that the effects of isotretinoin on sebaceous and eccrine glands are different. Morphometric studies on apocrine gland size in hidradenitis suppurativa during treatment with isotretinoin have not demonstrated a convincing change in gland size,[3] and clinical studies of efficacy have been disappointing[2] compared with the predictable effects on sebaceous gland structure and function. These studies, together with the results reported here, imply that the effects of retinoids on sebaceous, apocrine sweat and eccrine sweat gland function are quantitatively different.

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Bronchoconstriction due to 8-methoxypsoralen

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SUMMARY

A patient with psoriasis developed bronchoconstriction after oral 8-methoxypsoralen given as part of his photochemotherapy with PUVA. It was possible to treat the patient with PUVA whilst he was on prophylactic anti-asthmatic therapy. Twenty one further patients on photochemotherapy were studied in detail and none had evidence of impaired pulmonary function as a result of therapy.

The occurrence of bronchoconstriction after oral 8-methoxypsoralen (8-MOP), given as part of a photochemotherapy regimen, is rare, but five cases have been described.[1,2]

We report here a further case in a patient receiving PUVA for psoriasis, and also the results of a detailed study of 21 other patients receiving photochemotherapy.

CASE REPORT

A 57-year-old male computer programmer had had chronic plaque psoriasis for 40 years. He attended in October 1986 for his first course of photochemotherapy in the form of once weekly PUVA 'maintenance', his rash having been cleared by anthralin. He followed our conventional regimen of 8-MOP (Methoxsalen, Promedica) 0·5 mg/kg once per week 2 h before UVA exposure. He tolerated his first six PUVA treatments well, but on the day of his 7th treatment he developed a dry cough 5 h after taking 30 mg 8-MOP. The cough lasted 4 h and then abated. The 8th PUVA treatment a week later followed the same pattern. On the day of his 9th PUVA treatment he developed acute shortness of breath and wheeze 5½ h after taking the 8-MOP and his general practitioner diagnosed acute bronchoconstriction and treated him with nebulized salbutamol. His breathing returned to normal in 3 h.

There was no personal or family history of atopy, nocturnal cough, salicylate sensitivity or nasal polyps. He had smoked two cigarettes of rolled tobacco each day for the previous 20 years.

In between the three attacks described he remained well and did not require bronchodilator therapy. Physical examination was normal between attacks. In particular he had no evidence of cardiorespiratory disease. A chest X-ray was normal. Prick testing with our standard battery of

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allergens (house dust mite, cat fur, dog fur, horse hair, feathers and pollens B_2, B_3 and B_5) and 8-MOP in acetone were all negative and there was no evidence of peripheral blood eosinophilia. He had normal serum immunoglobulin levels.

The patient was admitted to hospital for further investigation which was undertaken in consultation with a respiratory physician. On 6 consecutive days off all therapy the patient measured his peak respiratory flow rate 2 hourly (using a Wright mini standard peak flow meter) and had formal spirometry 4 times daily. There was a marked diurnal variation, with a daily mean for the week of 473 l/min (expected value ± SD 561 ± 60 l/min) and a daily range of 445–530 l/min (Fig. 1); this is indicative of mild asthma.[3]

After a single dose of 30 mg 8-MOP with no subsequent UVA exposure his mean daily flow rate decreased to 290 l/min on that day and did not recover to pre-treatment levels until 6 days later (Fig. 1). His daily mean peak flow rate decreased to 393 l/min after one dose of 30 mg 8-MOP. His only symptom at this time was a dry cough which lasted 2–3 h on the day he received the 8-MOP. 8-MOP and one dose of salbutamol from his inhaler on day 1 were the only medication taken during this treatment period.

This sequence of events mirrored the history given previously, i.e. 5–5½ h after ingestion of 8-MOP he developed a cough and wheeze, and there was a concomitant decrease in peak expiratory flow rate. When the patient's psoriasis relapsed and failed to respond to conventional topical treatment, it was decided to try to clear his psoriasis with thrice weekly PUVA whilst he was on prophylactic anti-asthmatic therapy, consisting of beclomethasone dipropionate 500 μg (Becloforte®) twice daily.

Although his daily peak flow rate continued to exhibit asthmatic variability and slight dips after 8-MOP on PUVA treatment days (Fig. 2), he remained asymptomatic and his psoriasis cleared well on photochemotherapy. Both his PUVA and anti-asthmatic therapy were then stopped. At follow-up 3 months after clearance he remained well and had had no further asthmatic attacks.

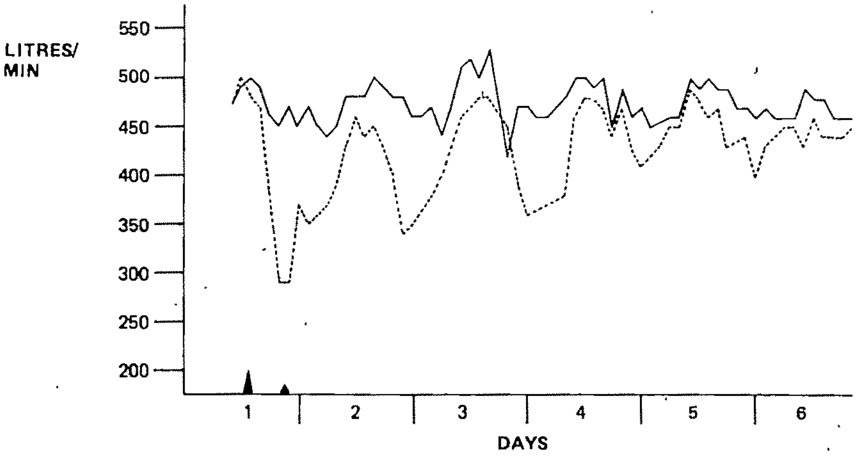

FIGURE 1. Two-hourly peak expiratory flow rates in the patient during a control 6-day period off all medication (——) and over 6 days after a single dose of 30 mg 8-MOP (- - - -). Large arrow head: dose of 8-MOP. Small arrow head: single dose of salbutamol from inhaler.

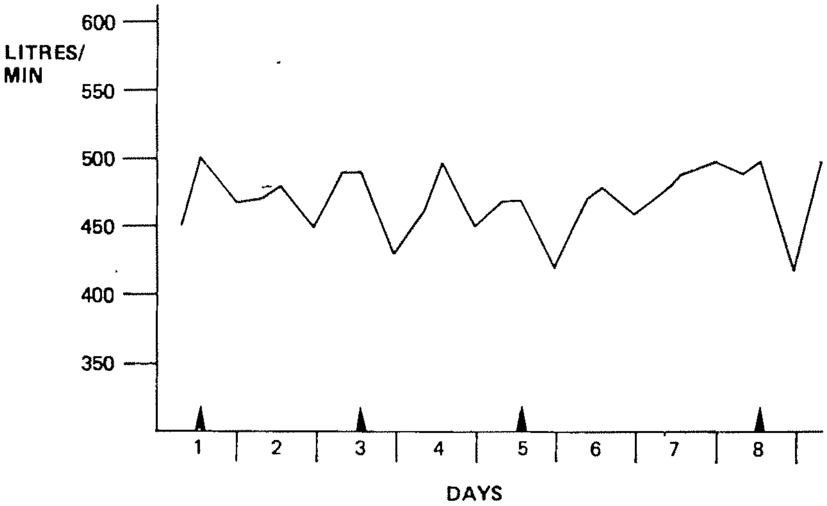

FIGURE 2. Eight-hourly peak expiratory flow rates in the patient during PUVA while on prophylactic beclomethasone dipropionate. Arrow heads indicate days on which PUVA was given.

CLINICAL STUDY OF 21 PATIENTS

To investigate whether this abnormal bronchial reaction was occurring asymptomatically in other PUVA patients and whether those with previous asthma were especially vulnerable, we studied a further 21 patients attending for photochemotherapy; eight gave a personal history of recent asthma; 13 had no history of atopy or asthma. In both groups peak expiratory flow rates were assessed 3 times daily during a control week (off all medication) and during a week in which they received their normal once-weekly maintenance dosage of 8-MOP and UVA.

There was no significant deterioration in daily mean peak expiratory flow rate during 8-MOP therapy in either the asthmatic or the non-asthmatic patients.

DISCUSSION

This case study showed that our patient, like those previously described, developed an adverse reaction to the oral psoralen preparation. Dragmole[4] raised the possibility of sensitivity to tartrazine being responsible for earlier cases, but this was ruled out in our patient because the 8-MOP tablets we use (Methoxsalen; Promedica) contain neither tartrazine nor benzoic acid.

That the reaction was due to the 8-MOP tablets and that UVA, including that normally present in the ambient lighting, was not necessary for its development, was shown by repeating the oral dose in the evening and keeping the patient in a room screened from extraneous UV; again, he developed a wheeze, 5 h after ingestion of 8-MOP.

The fact that symptoms developed 5 h after taking the 8-MOP is difficult to explain, but it is interesting that this was also the time lag in one of the patients reported by Wennersten.[2] Peak levels of the drug in the skin occur approximately 2 h after an oral dose, but this time is variable[5] and in some instances can be as long as 5 h; systemic bioavailability is also very variable, partly because hepatic first pass magnifies even small variations in absorption.[6] Animal experiments[7] have shown that distribution throughout the body was similar to that in the skin, although the lungs were not studied separately. In that study the tritium label used was still detectable 1 week after injection, so in spite of the short half life of the drug, sufficient may persist to cause the bronchoconstriction to last as long as it did in our patient.

The mechanism behind the bronchoconstriction is uncertain. We do not agree with Wennersten[2] that patients with pre-existing asthma are particularly at risk. Our patient had no history of asthma prior to taking 8-MOP. It was encouraging that he was able to tolerate photochemotherapy while using prophylactic anti-asthmatic medication and that he had no residual bronchoconstriction. The frightening and potentially serious bronchoconstriction after 8-MOP is apparently rare, but is something dermatologists prescribing PUVA should be aware of.

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Metastatic Crohn's disease responding to metronidazole

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SUMMARY

Metastatic Crohn's disease is a rare cutaneous manifestation of intestinal Crohn's disease, for which existing treatment is unsatisfactory. We report a case of perianal and vulval Crohn's disease which cleared completely on a 4-month course of metronidazole without adverse effects. The patient remained in remission 1 year later. The merits of long-term treatment with metronidazole are discussed.

Crohn's disease is a granulomatous inflammatory disease which can affect any part of the gastrointestinal tract from the mouth to the anus, but most commonly involves the terminal ileum. However, recent reports indicate that the process of Crohn's disease is not confined to the bowel, but may extend to sites remote from the gastrointestinal tract.[1] When such lesions are separated from Crohn's disease of the intestine by normal skin, they are referred to as metastatic Crohn's disease (MCD). These may represent 'skip lesions', more commonly found in the intestine, in which areas affected by Crohn's disease are interspersed with segments of apparently normal bowel.

Unfortunately, no ideal treatment has so far been reported for MCD, and the following methods have met with a variable degree of success: topical application of Milton solution, potassium permanganate solution, lotio rubra and steroid creams, intralesional triamcinolone, oral zinc sulphate, prednisolone, sulphasalazine and azathioprine, curettage, hemivulvectomy and radical vulvectomy.[2–5] We report here a case of MCD which responded dramatically to metronidazole.

CASE REPORT

A 45-year-old Indian lady presented in August 1983 with a 1 month history of a painful vulval swelling. There were no other symptoms related to the genital tract. However, over the previous 5 years she had noticed pruritus ani and rectal pain with bleeding on defaecation, but no abdominal pain. Her only medication was oral iron, administered for mild iron deficiency anaemia. The anaemia was discovered prior to an urgent haemorrhoidectomy performed in November 1982 for a prolapsed, thrombosed external haemorrhoid.

Examination of the vulva revealed a chronic, firm, diffuse, smooth swelling of the posterior

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aspect of the left labia majora (Fig. 1). There was no ulceration and vaginal examination was normal. Inspection of the perianal area showed an indurated anus with a chronic anterior fissure and a polyp. Sigmoidoscopy further disclosed a featureless distal 5 cm of rectum with a purulent exudate in the lumen.

Standard haematology and biochemistry were normal. A culture of the rectal exudate was sterile. Barium studies of the upper and lower bowel were normal apart from scattered diverticulae in the sigmoid colon. Histological examination of the excised anal polyp showed an extensive infiltrate of lymphocytes and plasma cells in the submucous and fibromuscular tissue. In addition, numerous non-caseating granulomata with giant cells were present in the dermis. Special stains and culture for acid fast bacilli and fungi were negative. A biopsy of the labial swelling also revealed non-caseating granuloma with giant cells (Fig. 2), and was likewise negative for acid fast bacilli and fungi. A chest X-ray was clear and a Mantoux test (10 IU) moderately positive.

Treatment with oral prednisolone, sulphasalazine suppositories and a high fibre diet failed to make an impression on the perianal or vulvar lesions. Later, oral metronidazole 800 mg daily was introduced and after 4 months there was complete symptomatic and objective healing, and this was maintained for 1 year after stopping the drug.

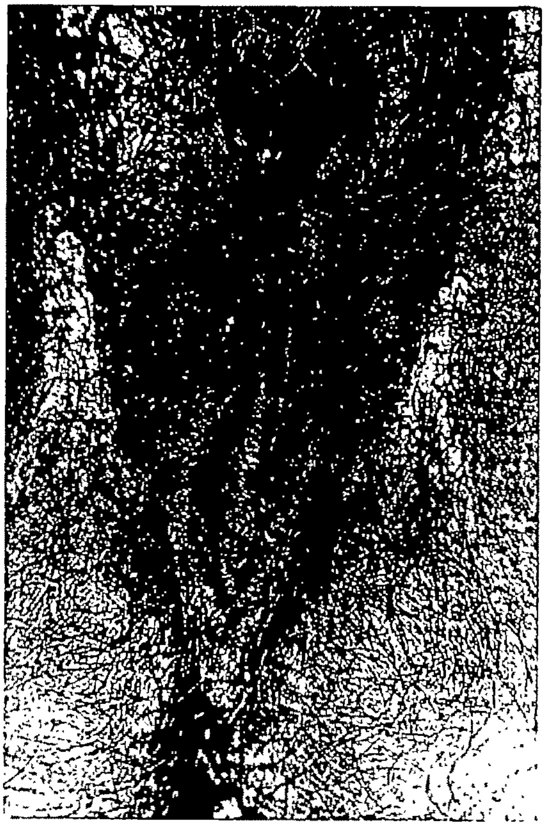

FIGURE 1. A diffuse swelling of the left labia majora.

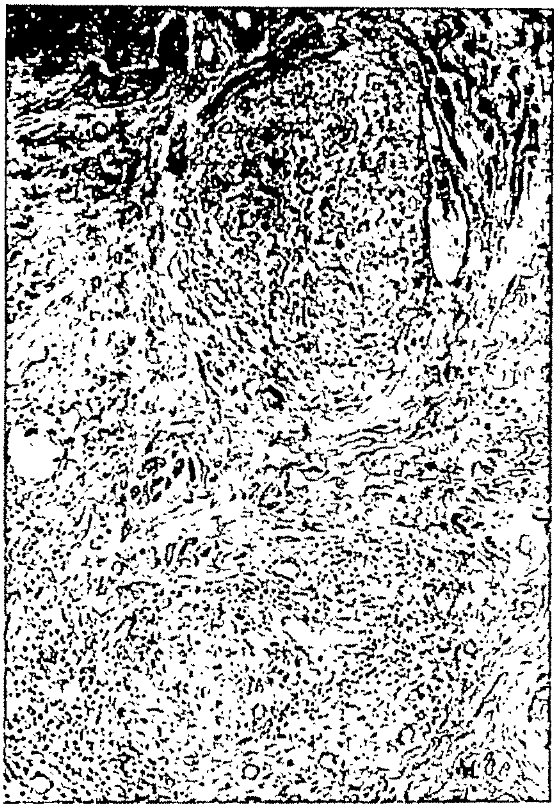

FIGURE 2. Vulval biopsy showing epithelioid cell granuloma (H & E; original × 32).

DISCUSSION

The cutaneous manifestations of Crohn's disease are present in 22–44% of cases.[6–7] The commonest lesions are perianal and peristomal ulcers, as well as fistula and abscess formation. Vascular reactions such as pyoderma gangrenosum, erythema nodosum and cutaneous polyarteritis nodosa are rare, as are the skin changes of malabsorption, psoriasis, vitiligo, epidermolysis bullosa aquisita and erythema multiforme.[8] MCD is extremely rare with less than 20 cases documented in the literature.[8–9] The lesion of MCD may manifest as an ulcer, nodule or plaque and has been identified on the face, retroauricular region, upper and lower extremities, infra-mammary area, umbilicus, lower abdominal wall, penis and vulva.[8]

To establish a diagnosis of MCD it is necessary to demonstrate histologically a non-caseating, giant cell granuloma occurring at a site distant from the gastrointestinal tract in a patient with intestinal Crohn's disease. It is also necessary to exclude other granulomatous diseases such as sarcoidosis, tuberculosis, fungal infection and foreign body reaction which might occur coincidentally with Crohn's disease.[10] A biopsy specimen should, therefore, be sent for special stains and culture for fungi and acid fast bacilli, and a chest X-ray and tuberculin test are desirable.

Brandt *et al.*[11] convincingly demonstrated the value of metronidazole in perineal Crohn's disease unresponsive to other medication. They used a dose of 20 mg/kg daily for 3 to 36 months and found complete or advanced healing in all 26 patients treated. This prompted us to use metronidazole for our patient who had perianal disease in addition to vulval swelling, and the result after 4 months of treatment was complete healing of both lesions. It is not surprising that both lesions healed simultaneously as they share a number of common features: they are both usually associated with colonic Crohn's disease and seldom with Crohn's disease affecting the small bowel alone.[10] They are independent of intestinal disease activity and may deteriorate even after adequate control of intestinal disease with medical measures or surgical resection.[8,12] They are unresponsive to conventional therapies such as sulphasalazine, steroids and broad spectrum antibiotics,[12] and most cases of MCD that have been reported have also had perianal Crohn's disease.[10] However, the relationship between these lesions of Crohn's disease is not straightforward, as perianal involvement in Crohn's disease is relatively common, whereas metastatic lesions are distinctly rare.[13]

The exact mechanism of action of metronidazole in Crohn's disease remains to be elucidated, but its antimicrobial, anti-inflammatory and immunosuppressive properties may be of significance.[14] In one study this drug was found to suppress granuloma formation around the eggs of *Schistosoma mansoni* injected into the pulmonary microvasculature and it was postulated that it inhibited selective aspects of cell-mediated immunity.[15] However, this is unlikely to explain its entire effect, as Bernstein *et al.*[12] were unable to demonstrate a significant alteration in cell mediated immunity despite the beneficial effect they observed with metronidazole.

The prolonged administration of metronidazole is associated with a number of adverse effects. The most important symptom is bilateral pedal parasthesia which occurred in 50% of patients in one study using metronidazole 1000–1500 mg daily.[11] This started approximately 6 months after initiation of therapy, was responsible for the withdrawal of six patients from the trial, and was dose-dependent as it disappeared after reducing the dose or stopping the drug. This predominantly distal sensory polyneuropathy has been shown by electron microscopy to be due to peripheral axonal degeneration of both myelinated and unmyelinated fibres, probably induced by inhibition of neuronal protein synthesis.[16] Other side-effects include a metallic taste, darkening of urine, nausea, anorexia, fatigue, headache, glossitis, urticaria and reversible neutropenia.[12]

Metronidazole 800 mg daily was found to be effective in healing MCD in our patient, and was without any serious adverse effects. As recommended for perineal Crohn's disease, treatment for MCD should continue for at least 4 months and the failure to observe this requirement explains why metronidazole has been found ineffective by some authors.[2]

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Hyperimmunoglobulin E syndrome: Treatment with isotretinoin

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SUMMARY

A patient with hyperimmunoglobulin E syndrome is described. Recurrent cutaneous staphylococcal abscesses were a prominent feature and had been resistant to treatment with antibiotics and surgical drainage. Following a 4-month course of treatment with isotretinoin, he developed no further abscesses over a 6-month follow-up period. Detailed immunological investigations were undertaken before and after treatment and no change was found to account for his improvement. We consider that the beneficial effects of isotretinoin in this situation are likely to be due to a reduction in sebaceous gland size and activity, rather than to any effect on the underlying disorder.

Hyperimmunoglobulin E syndrome is characterized by persistently elevated serum levels of IgE, an eczematous rash, and a tendency to develop recurrent infections, particularly of the skin (cold abscesses) and respiratory tract.[1] An abnormality of neutrophil chemotaxis has been described in some patients with this condition.[2,3] There have been various approaches to the treatment of this disorder, but none has been consistently successful. Immunostimulation by the use of levamisole has been shown to be ineffective in one well controlled study.[4] Repeated surgical excision of cutaneous abscesses and extended courses of antibiotics are also unsatisfactory and do not influence the natural history of the condition. We report here a patient with this syndrome whose staphylococcal skin abscesses responded dramatically to a 4-month course of treatment with a systemic retinoid, isotretinoin (Roaccutane®, Roche). This is the first report, to our knowledge, of the use of systemic retinoids in this condition.

CASE REPORT

The patient was a 15-year-old boy. He had been delivered at 37 weeks gestation after a normal pregnancy, birth weight 5lbs 9ozs (2·56 kg). No abnormalities were noted at birth.

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At the age of 10 months he was admitted to hospital with mycoplasma pneumonia, and made an uneventful recovery. At this time he was noted to have a rash on his face and scalp which was initially thought to be infantile seborrhoeic dermatitis, although the diagnosis was later revised to one of eczema. Over the next few years he continued to have episodes of bacterial pneumonia and had repeated admissions to hospital. During one episode of respiratory infection he developed a lung abscess. Sputum culture usually grew *Staphylococcus aureus*, but *Haemophilus influenzae* was also occasionally isolated. His eczematous rash gradually deteriorated, with frequent episodes of secondary bacterial (staphylococcal) infection. Asthma developed at the age of 6 years and at 11 years he had a single episode of osteomyelitis of the ankle, although no organism was isolated. He also suffered from recurrent meibomian cysts which required surgical drainage; culture of the contents of these cysts consistently grew *Staphylococcus aureus*.

Over the next few years he developed repeated cutaneous staphylococcal abscesses, especially over the back and chest, and in the axillae. These were treated with numerous courses of antibiotics, and surgical drainage where necessary. This problem persisted and prompted his referral to our department for further treatment.

On examination, he was noted to have the coarse facies and broad nasal bridge said to be typical of patients with hyperimmunoglobulin E syndrome[5] (Fig. 1). Both parents were Caucasian. There was a widespread eczematous eruption of a papular type, principally affecting the forearms, presternal area and thighs. The distribution and morphology of the rash was not typical of atopic eczema, and there was no family history of atopy. In addition, there were numerous fluctuant non-inflamed abscesses on the back, neck and chest, with prominent scars from previous surgical excisions of similar lesions (Fig. 2).

It was noted that the cutaneous abscesses were chiefly located in areas where sebaceous glands were most numerous. Furthermore, the problem of recurrent abscesses had developed during puberty, at a time when sebaceous gland activity is increased. On the basis of these observations, he was treated with a 4-month course of isotretinoin, 1 mg/kg/day (60 mg/day), as this drug is known to modify sebaceous gland size and activity.

Treatment was well-tolerated, although there was a noticeable worsening of his eczema after 2 months, which responded to a temporary increase in potency of the topical steroid he was using. At the end of the treatment period, no further abscesses were present (Fig. 3), and this improvement had been maintained for a period of 6 months at the time of writing. There was also a noticeable improvement in his eczema after treatment was discontinued and he was able to reduce both the potency and amount of topical steroid used.

Investigations

Staphylococcus aureus was consistently isolated from the abscesses, and was resistant to penicillin, but sensitive to a number of other antibiotics including flucloxacillin and erythromycin. Apart from a persistent eosinophilia, the blood count was normal and the patient was not neutropenic.

Radioallergosorbent tests (RAST) were positive to mixed grasses (2 +), house dust mite (2 +) and mixed moulds (1 +).

A number of immunological investigations were performed before and after treatment with isotretinoin. Immunoglobulin levels were measured by radial immunodiffusion and IgG subclasses by simple immunodiffusion with monoclonal antibodies. Antibodies to the commensals *E. coli* and *Candida albicans* were measured respectively by haemagglutination[6] and immunofluorescence.[7] Antibodies to *Staphylococcus aureus* were measured by an ELISA

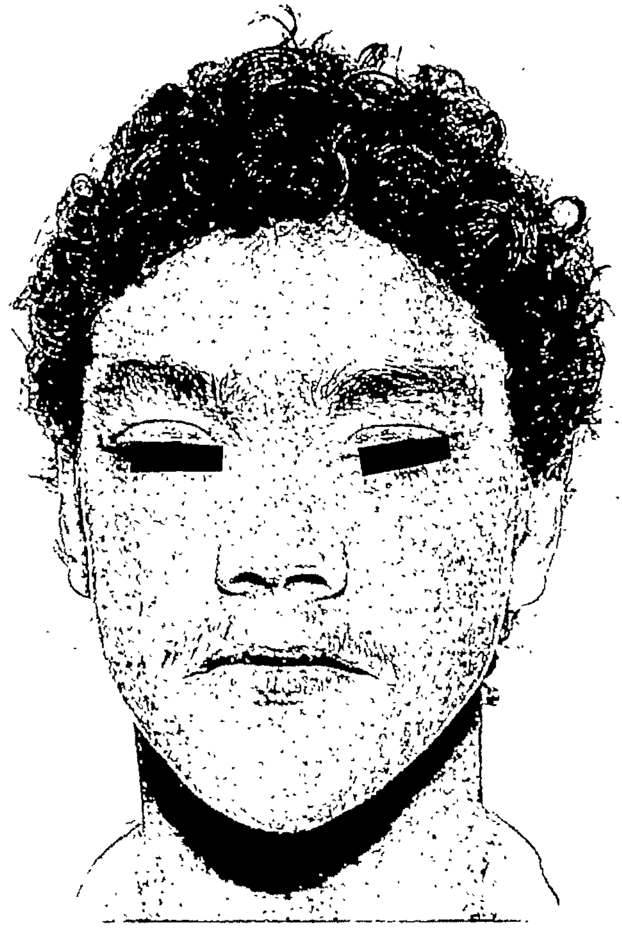

FIGURE 1. Patient with hyperimmunoglobulin E syndrome showing characteristic appearance with a broad nasal bridge and coarse facies.

method[8] with alkaline phosphatase-conjugated anti-IgG or -IgE and a protein A-deficient strain of *S. aureus* (Wood strain 46).

Haemolytic complement was assayed using rabbit erythrocytes (alternative pathway) or antibody-coated sheep erythrocytes (classical pathway) in microtitre trays with quantitation by means of an ELISA reader. Nitro-blue tetrazolium reduction by neutrophils was assessed on whole blood stimulated by endotoxin. For chemotaxis, neutrophils were purified by a 2-layer gradient centrifugation and tested by an under agarose method with the chemotaxin N-formyl-L-methionyl-L-leucyl-L-phenylalanine. Staphylococcal phagocytosis and intracellular killing were measured as described by Murphy *et al.*[9]

T cell subsets were determined using a fluorescent activated cell sorter (FACS) after labelling with the appropriate monoclonal antibodies. For measurement of interferon (IFN) production, lymphocytes were stimulated with either Sendai virus (alpha-IFN) or staphylococcal enterotoxin (gamma-IFN). Interferon activity was assayed as previously described.[9]

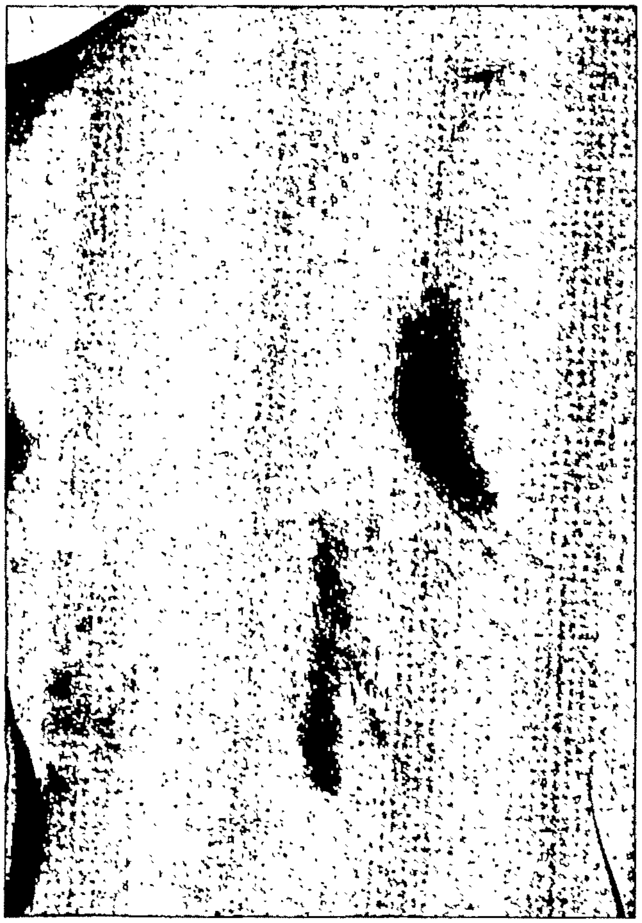

FIGURE 2. Appearance of patient's back before treatment. Numerous abscesses are present and scars from previous excisions of abscesses can be seen.

RESULTS

Numerous investigations were performed over a 5-year period without revealing any evidence of immunodeficiency. In particular, immunoglobulin concentrations were normal (apart from a raised level of IgE, which was consistently greater than 5000 IU/ml), and antibodies to the commensal organisms *E. coli* and *C. albicans* were present, indicating normal antibody producing capacity. IgG antibodies to *S. aureus* were present at a normal titre, but IgE antibodies to the organism could not be detected (in contrast to some patients with hyper-IgE syndrome[10]). Complement activity was readily detectable by haemolytic assays, and neutrophil chemotaxis, phagocytosis and bactericidal activities were unimpaired. Lymphocytes were present in peripheral blood in normal numbers and these cells produced interferon normally.

Results of tests of immunological function before and after treatment with isotretinoin are listed in Table 1. Before treatment the classical pathway complement level was slightly reduced and the alternative pathway complement level was raised (probably as a result of staphylococcal

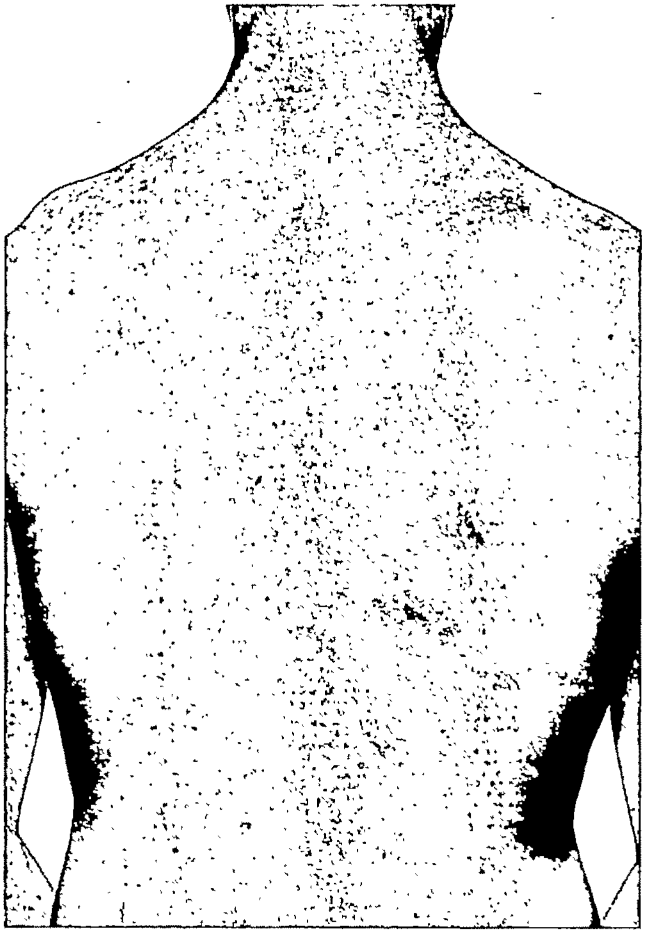

FIGURE 3. After treatment. The cutaneous abscesses have resolved completely.

infection). After treatment and with resolution of the cutaneous infection, complement levels reverted to normal. The only other change noted was a reduction in the T8 cytotoxic/suppressor subset; we are uncertain of the significance of this finding.

DISCUSSION

The criteria on which a diagnosis of hyperimmunoglobulin E syndrome can be made have recently been summarized.[5] Constant features are recurrent bacterial infections of the skin and sinopulmonary tract, and the presence of serum IgE levels which are at least 10 times normal (> 2000 IU/ml). Variable features (not all of which may be found in any one patient), include coarse facies, eczematous rashes, cold cutaneous abscesses, mild eosinophilia, and a defect in neutrophil chemotaxis. Although our patient had no identifiable abnormality of neutrophil function, there is no doubt that he fulfilled the clinical criteria for the diagnosis of hyperimmunoglobulin E syndrome, and the other laboratory data would support this. Buckley

TABLE 1. Results of immunological investigations before and after treatment with isotretinoin

		Before treatment	After treatment	Normal range
Immunoglobulins	IgE IU/ml	> 5000	> 5000	< 100
	IgG g/l	12·2	13·3	5–14
	IgA g/l	1·8	1·4	1–4
	IgM g/l	1·8	2·0	0·5
IgG subclasses:		all present	all present	
Anti-*E. coli* antibody titre		1:256	1:256	> 1:32
Anti-*C. albicans* antibody titre		1:32	1:32	> 1:8
Complement	Classical CH_{50} U/ml	750	1300	1000–1500
	Alternative CH_{50} U/ml	470	170	80–200
Neutrophils	NBT reduction	normal	normal	
	Chemotaxis	normal	normal	
Lymphocytes	T4 (helper)	0·8 × 10^9/l	0·9 × 10^9/l	> 0·75 × 10^9/l
	T8 (cytotoxic)	0·6 × 10^9/l	0·2 × 10^9/l	> 0·3 × 10^9/l
	alpha interferon	160	160	> 20
	gamma interferon	32	32	> 8

and Becker[11] have emphasized that hyperimmunoglobulin E syndrome is 'not defined by the presence or absence of either a polymorphonuclear or mononuclear chemotactic defect.' It seems most likely that the mechanism of action of isotretinoin in our patient was through its effect on the pilosebaceous gland. Treatment with isotretinoin results in a marked reduction of both sebum excretion rate and dermal (sebaceous) lipogenesis,[12,13] and a reduction in sebaceous gland mass.[14,15] Other studies have shown that this drug has no effect on bacterial growth, and the changes seen in ductal flora during treatment are the result of changes in the microenvironment.[16,17] It may be that the improvement in our patient was due to an anatomical reduction in the sites available for colonization by bacteria rather than a change in the abnormality of immune function. Although isotretinoin has been shown to be a potent inhibitor of both neutrophil lysosomal enzyme release and superoxide anion production *in vitro*,[18] the significance of this mechanism is uncertain where the inflammatory component (in this case, of the abscesses) is already minimal.

We feel that this novel approach to treatment warrants further investigation.

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Epidermolysis bullosa acquisita associated with vesicular cystitis

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SUMMARY

A 55-year-old woman with epidermolysis bullosa acquisita (EBA) who had associated vesicular cystitis is described. The clinical, histological and immunohistological features of the skin and mucous membranes were in accordance with previous reports of this disease. The patient also had symptoms of cystitis, and a cystoscopic examination showed areas of erosions and vesicles in the wall of the urinary bladder. Histological and immunopathological studies of the urinary bladder demonstrated sub-mucosal vesicles, and linear deposits of IgG and C_3 along the mucosal–lamina propria junction. These bladder changes may be induced by the autoantibodies present in EBA.

Epidermolysis bullosa acquisita (EBA) is a rare autoimmune disease in which mechanobullous lesions develop in the skin and mucous membranes. Patients with EBA usually develop vesiculobullous, erosive and ulcerative changes on the extensor surfaces of the joints and other points of mechanical stress, including oral, oesophageal, genital, anal, and ocular mucosae.[1–3] The blister is formed by separation of the skin or mucous membrane at the basement membrane zone (BMZ) between the epidermis and the dermis or between the mucosa and the lamina propria. Recently, Yaoita *et al.*,[4] and Nieboer *et al.*,[5] demonstrated linear deposits of IgG in the BMZ of skin from EBA patients, which suggested that blistering may be initiated by an immune process.

There were some problems in diagnosing this disease and distinguishing it from other bullous disorders. Roenigk and Pearson[1] established the diagnostic criteria for this disease as, (1) trauma induced bullae, (2) onset after infancy, (3) no family history, and (4) exclusion of other bullous diseases by confirming the presence of deposits of IgG containing immunoreactants in the sub-basal lamina anchoring fibril zone of the basement membrane.

We describe here a patient with EBA who had a long history of cystitis and in whom we found unusual mucosal changes in the urinary bladder.

CASE REPORT

A 55-year-old Korean woman presented with a 7-year history of skin fragility characterized by recurring, non-painful blisters at sites of trauma, especially over joints, and some spontaneous

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pruritic blisters occurring on the arms. These bullous lesions resolved with scarring. Most of the bullous lesions were related to trauma at work and were not related to sun exposure, medications, diet, or any other illnesses. Since the onset of her skin disease and she had taken various corticosteroids and antibiotics to suppress new lesion formation. She had also suffered from a moderate degree of intermittent urinary frequency and tenesmus for about 5 years. Her personal and family histories were unremarkable.

Examination showed a thin, 45 kg, normotensive woman with skin lesions on the trauma prone areas of the body including the dorsal aspects of both hands and feet, wrists, elbows, ankles, and knees. New lesions were 3–15 mm tense vesicles and bullae on non-erythematous or erythematous skin. The bullae contained a clear or slightly haemorrhagic fluid. Resolving lesions were dry and crusted, or erosive with occasional bleeding, and left multiple superficial atrophic scars, hypo- or hyperpigmentation, and prominent milia. Distal onycholysis was noted on several finger nails. Cicatricial alopecia was seen on the scalp, axillae, and the pubic areas. Multiple discrete oral erosions, vesicles, and ulcerative changes, especially on the lateral aspect of the tongue and the soft palate were noted. The vulval and perianal skin and mucous membranes showed several erosive patches; however, no ulcerative changes were found. Other physical findings were normal.

Oesophagoscopy revealed a few patchy erosions and signs of oesophagitis. Gastroscopic examination of the stomach revealed no abnormalities. Barium X-rays of the upper gastrointestinal tract and small bowel follow-through were normal. Sigmoidoscopy of the colon showed two areas of hyperaemic mucosa, but there was no evidence of erosions or ulcerative changes. Cystoscopic examination of the urinary bladder revealed several foci of erosive changes and discrete pebble-like vesicular lesions, especially on the lateral walls and on the bladder neck. Multiple pseudopolyps were seen around the erosive and vesicular areas. Both ureteral orifices were normal in position and shape, and normal efflux of clear urine was found on contraction. The bladder capacity was not reduced. An intravenous pyelogram revealed no abnormalities.

Laboratory investigations

A full blood count and differential count, platelet count, serum electrolytes, and examination of stools for occult blood were normal or negative. The ESR was elevated at 50 mm/h. Routine urinalysis revealed many white blood cells, and a few red blood cells were found on re-examination. Urine cultures showed no significant growth. A chest X-ray and an electrocardiogram were unremarkable. Serum protein electrophoresis showed an increased gamma globulin level, but a monoclonal IgM-band was not detected. The serum IgG level was increased at 2600 mg/dl, but other immunoglobulin levels were within the normal ranges. Serum levels of total haemolytic complement (CH_{50}), C_3 and C_4, anti-nuclear antibody, late-fixing rheumatoid factor, cryoglobulins and alpha-antitrypsin, the fasting blood glucose level, liver and thyroid function tests, blood levels of urea nitrogen and creatinine, 24 h urinary creatinine clearance ASTO titre, VDRL, urinary Bence-Jonce protein, urinary porphyrin, and an NBT (nitro-blue tetrazolium) test were all normal or negative. Serum C-reactive protein was strongly positive. The proportion of T cells in the peripheral blood, measured by monoclonal antibody techniques was 54% (normal, 55%–70%).

Histological, immunopathological and electron microscopic studies

Histology of biopsies from slightly erythematous perilesional skin on the extensor forearm and from the buccal mucosa showed a subepidermal blister containing red blood cells and neutrophils. A moderate perivascular inflammatory infiltrate consisting of neutrophils, eosinophils, and lymphohistiocytic cells was seen in the upper dermis and lamina propria. Crystal violet and congo red stains were negative for amyloid. Direct immunofluorescent (IF) studies were performed using standard methods,[6] on perilesional and clinically normal skin, and on buccal mucosa, and showed linear deposits of IgG and C_3 at the BMZ of all three specimens, however, IgA, IgM, and IgE were not detected. Indirect IF studies using the patient's serum, with intact normal human skin taken from the inner thigh as the substrate, demonstrated circulating anti-BMZ IgG antibodies at a titre of 1:80, and anti-basal cell cytoplasmic IgG antibodies at a titre of 1:16. Indirect IF studies using sodium chloride split-skin preparations as the substrate were also performed,[7,8] and the serum anti-BMZ IgG was found to bind only to the dermal surface below the plane of separation. On immunoelectron microscopy, carried out as previously described,[5] the deposition of IgG was observed just beneath the basal lamina in the dermis of both perilesional and clinically normal skin.

A cytoscopic biopsy specimen taken from a perilesional area of the bladder wall showed sub-mucosal vesicles separating the mucosal epithelium from the lamina propria with a mild infiltrate of neutrophils, eosinophils, and lymphohistiocytic cells beneath (Fig. 1). Vascular dilatations and stromal oedema were also noted. Deeper parts of the specimen were histologically normal. In addition, somewhat disconnected linear deposits of IgG and C_3 could be seen along the mucosal-lamina propria junction, at and around the vesicular lesions in frozen tissue examined using an indirect immunoperoxidase technique[9] (Fig. 2).

Treatment and course

The patient was instructed to avoid trauma and started prednisolone (40 mg/day), dapsone (75 mg/day), and antibiotics for the prevention of secondary bacterial infections in the skin and urinary bladder. Topical therapy was also given for her skin and mucous membrane lesions. After 5 weeks of treatment, blistering of the skin and mucous membranes and the bladder symptoms had subsided considerably. Serum titre of anti-BMZ antibodies had falled to 1:20. A cystoscopic examination of the bladder showed only pseudopolyps around the previously lesional areas. Results of urinalysis returned to normal.

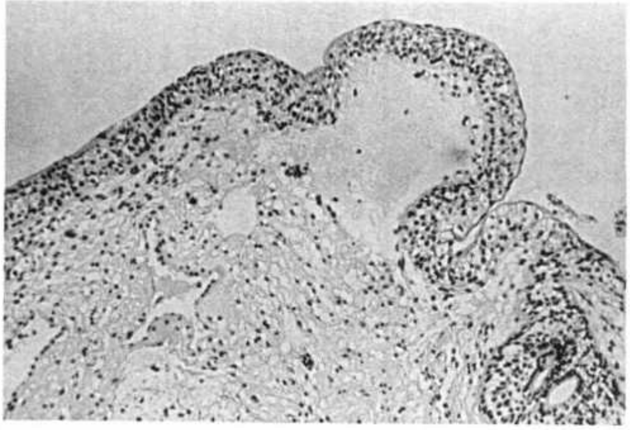

FIGURE 1. Involved urinary bladder wall, showing a sub-mucosal vesicle and a mild stromal infiltrate consisting of neutrophils, eosinophils, and lymphohistiocytic cells. Vascular dilations and stromal oedema can also be seen (H&E, original × 100).

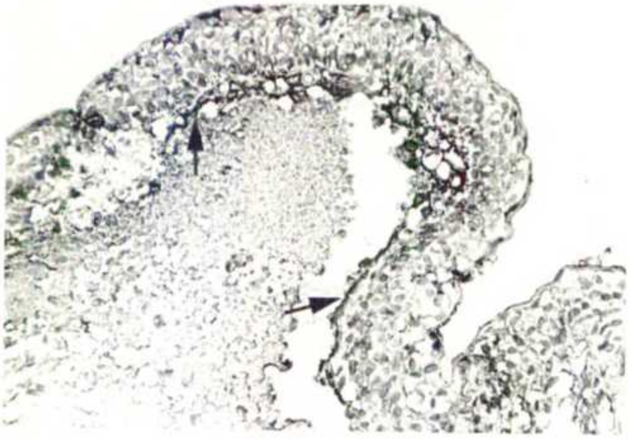

FIGURE 2. Indirect immunoperoxidase staining of a vesicular lesion in the urinary bladder, showing somewhat disconnected linear deposits of IgG (arrows) along the mucosa–lamina propria junction. (Counterstained with haematoxylin; original × 200).

DISCUSSION

EBA is frequently associated with other systemic diseases, usually of an autoimmune nature, such as inflammatory bowel disease, systemic lupus erythematosus, amyloidosis, chronic thyroiditis and diabetes mellitus.[10] However, symptoms or signs of cystitis suggesting primary pathological changes in the urinary bladder have not previously been reported in cases of EBA to our knowledge.

The pathogenesis of EBA appears to be complex. Immunopathologically this blistering disease is characterized by the presence of circulating complement binding IgG anti-BMZ antibodies and linear deposits of IgG and complement at the sub-basal lamina of lesional as well as clinically normal skin.[4,5,7] These features suggest that antibody-mediated complement activation may play a role in the pathogenesis of inflammation and dermo-epidermal separation in this disease.[10,11] Recently, Woodley[12] suggested that another possible pathway producing fragile skin and mucous membranes could be through EBA autoantibodies abrogating the bond between the EBA antigen (probably type VII procollagen[13]) and fibronectin, which normally plays a role in dermo-epidermal adherence in the sub-basal lamina region of the dermo-epidermal junction.

Whatever the mechanisms, the extent of the skin and mucosal lesions will be confined to areas containing EBA antigen. The anatomical distribution of the EBA antigen has been studied by Furue[14] and Paller *et al.*,[15] who showed that the antigen is limited to the skin, and the oral, oesophageal, tracheal, genital and anal mucosae, and the cornea. The presence of this antigen in the urinary bladder is controversial, however, Furue *et al.*[14] reported weak positive results for EBA antigen in the bladder using indirect IF methods with high titre EBA serum. The amounts of this antigen may vary in different tissues. The presence of the antigen in the skin and oral mucosa seems more easily detectable immunopathologically, perhaps due to variation of the amount of the antigen in different tissues, and this might be relevant to the clinical manifestations of the disease. In the urinary bladder EBA antigen appears to be expressed only minimally, and there may be variation in this between individuals.

The clinical features of EBA are heterogeneous.[16] During the inflammatory phase the lesions can resemble those of classical bullous pemphigoid. In our patient who had spontaneously occurring blisters in the skin and supposedly in the urinary bladder, with some cellular infiltrates, the inflammatory and the non-inflammatory phases seemed to occur in combination.

The evidence for the urinary bladder lesions being a manifestation of EBA in our patient are, firstly, the unusual cystoscopic findings of erosions and areas of pebble-like vesicles; secondly, the histological similarity between the skin and the urinary bladder lesions with sub-epithelial blister formation; thirdly, the linear deposits of IgG and C_3 at the mucosal–lamina propria junction found in the cystoscopic biopsy, and, fourthly, the parallel course of improvement in the cystoscopic findings in the urinary bladder and the lesions of the skin and mucous membranes during treatment.

The cystoscopic findings, and histological, and immunopathological features of the urinary bladder in our patient could be considered to be a type of interstitial cystitis.[17] Although further histopathological investigations are required to determine the nature of the urinary bladder abnormalities in patients with EBA, I believe that the bladder changes seen in the present patient were caused by the EBA disease process. Perhaps, more attention should be paid to vesicular pathological changes of the urinary bladder in patients with EBA.

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Cutaneous nodular sarcoidosis with granulomatous renal sarcoid

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SUMMARY

We describe a 66-year-old lady who presented with tender nodules on her trunk and limbs which were histologically proved to be sarcoid. Following steroid therapy the skin lesions remitted, but renal failure developed due to a severe granulomatous interstitial nephritis. Clinical and biochemical improvement followed treatment with high dose steroids, although renal function and renal biopsy remained abnormal.

Sarcoidosis of the skin is frequently associated with histological involvement of other tissues including the kidney. However, renal failure secondary to sarcoid granulomata is exceptionally rare.[1] We report a case of sarcoidosis in which renal failure occurred at a time when the dermatological features were in remission.

CASE REPORT

A 66-year-old Iranian lady, who had lived in the U.K. for 10 months, presented with tender, nodular lesions on her back, trunk, arms and legs, which had developed over a 2-week period following a 'flu-like illness' for which she has been given tetracycline and amoxycillin for 1 week each. She also complained of gritty eyes, muscle pains and evening pyrexia.

There was no significant past medical history or family history of note.

On examination multiple tender, erythematous, nodular lesions were present over her back, chest, abdomen and limbs. The epitrochlear nodes were enlarged. She had a papillary conjunctivitis and swelling of the parotid and submandibular glands. Pitting oedema of the ankles was present.

Investigations

The ESR was 55 mm in the 1st hour. A full blood count was within normal limits apart from a platelet count of 612×10^9/l. Serum angiotensin converting enzyme was raised at 51 U/l (normal 9–35 U/l). Serum creatinine, urea and electrolytes, liver function tests and serum calcium were normal. Sputa and early morning urine specimens were negative for acid fast bacilli. She had no reaction to a Mantoux test of 10 units of tuberculin intradermally. A Kveim test was also negative. Chest X-ray and pulmonary function tests showed no evidence of sarcoidosis.

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Skin biopsy of one of the lower back lesions revealed multiple non-caeseating granulomata throughout the dermis and in the subcutis. These consisted of epithelioid cells and some multinucleated giant histiocytes. No organisms were identified.

A diagnosis of cutaneous sarcoidosis was made.

Progress

She was started on 40 mg prednisolone daily and her symptoms, pyrexia and lesions began to resolve within 4 days. The dose was then reduced gradually and she remained asymptomatic on prednisolone 10 mg daily for 6 months; however, when the dose was decreased further skin lesions recurred on the legs. One year after presentation the corticosteroid was stopped and she remained free of lesions for a further 4 months. At that time she started to complain of muscle cramps. One skin lesion appeared on her forehead.

She was found to have a normochromic normocytic anaemia with haemoglobin 9·3 g/dl, ESR 80 mm in the 1st hour, plasma urea 12 mmol/l and creatinine 249 mmol/l. Serum calcium, ionised and non ionised, and serum phosphate were normal. Alkaline phosphatase was elevated at 170 U/l and 25-hydroxy vitamin D was decreased at 7 μmol/l (normal range 15–60 μmol/l in the winter). Over 10 days her plasma urea rose to 34 mmol/l and creatinine to 321 mmol/l.

A renal biopsy showed a very severe granulomatous interstitial nephritis, with numerous epitheloid granulomata in all parts of the cortex and medulla (Fig. 1).

In view of her deteriorating renal function, treatment with i.v. methylprednisolone 500 mg daily for 3 days was started, followed by oral prednisolone 40 mg daily. Renal function improved rapidly initially, but 4 months later when she was still taking prednisolone 25 mg daily her plasma creatinine was still > 200 mmol/l.

DISCUSSION

Renal failure secondary to sarcoidosis is uncommon.[2] The most frequent underlying cause is hypercalcaemia[3] thought to be due to an enhanced sensitivity to vitamin D. Renal failure may

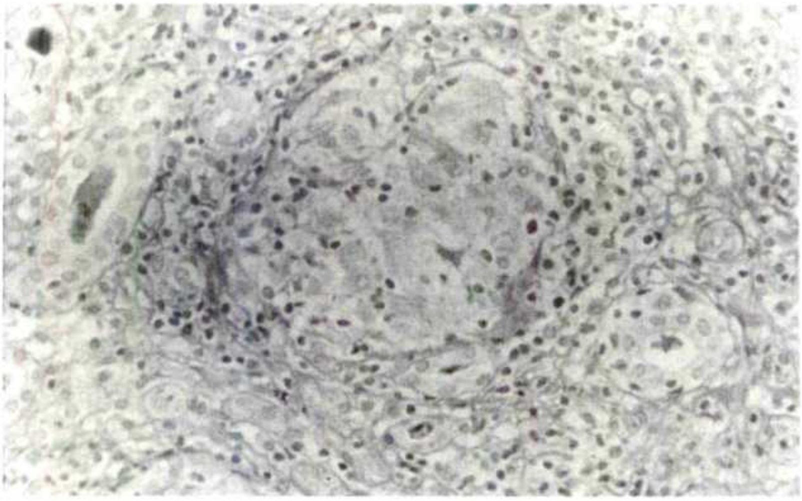

FIGURE 1. Renal biopsy, showing a very severe granulomatous interstitial nephritis, with epithelioid granulomata. (Original × 300).

also occur as a result of nephrocalcinosis in the absence or presence or hypercalcaemia.[4] Glomerulonephritis in sarcoidosis has also been reported as causing renal failure.[3]

Granulomatous infiltration of the kidneys causing renal failure, as in our case, is exceptionally rare. The incidence of granulomatous infiltration is unknown as many patients remain asymptomatic, in which case a renal biopsy is unlikely to be taken. In one series of renal biopsies, of presumably selected cases of sarcoidosis, granulomata were found in 40% of cases.[3] In 7–27% of patients with sarcoidosis renal granulomata are evident at autopsy.[5]

The response of our patient to corticosteroid therapy was initially good. However, her renal function failed to return to normal. In previously reported cases a gradual decrease in renal function with hyaline glomerulofibrosis has been described despite repeated courses of corticosteroids.[6,7] The prognosis in our case must, therefore, be cautious and it is expected that she will eventually require dialysis or renal transplantation.

This case illustrates a rare life-threatening manifestation of sarcoidosis associated with a milder cutaneous form.

ACKNOWLEDGMENTS

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Sudoriparous angioma—regression following intravascular aethoxysclerol, a sclerosing agent

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SUMMARY

We report a case of sudoriparous angioma on the dorsum and plantar aspect of the left foot which increased in size on standing, and which was accompanied by sweating in the overlying epidermis and throbbing pain. The lesion regressed considerably following intravascular injection of 1% aethoxysclerol.

Sudoriparous angioma presents with spontaneous pain and sweating following pressure.[1,2] Dilation of vascular spaces and hyperplasia of sweat glands has been reported in sudoriporous angioma.[1,2] Localized hyperhidrosis is known to accompany conditions such as subclavian aneurysm,[3] osteoma of vertebrae[4] and blue rubber bleb naevus.[5]

We report a case of sudoriparous angioma in which significant remission was obtained following treatment with a sclerosing agent.

CASE REPORT

An 11-year-old girl presented with a swelling over the left foot which had started as a small lesion when she was 3 months old and had gradually increased in size (Fig. 1). She also complained of a throbbing pain which was more marked on lowering the legs and on standing.

A soft, compressible, irregular non-tender swelling, 11 cm × 5 cm was seen on the dorsum of the left foot, and a similar swelling on the plantar aspect of this foot. The lesion, which was covered by hyperpigmented skin was seen to decrease in size on raising the leg and to increase in size following lowering of the leg. The increase in size was accompanied by throbbing pain and sweating in the overlying epidermis. The presence of sweat was confirmed by violet discolouration of starch power applied after iodine had been applied and allowed to dry.

X-rays showed soft tissue swelling without bony involvement. Routine blood tests showed no abnormalities. As the lesion was extensive and highly vascular a biopsy was not attempted. A

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phlebogram showed venous malformation with numerous feeder vessels from both the dorsum and the sole of the foot (Fig. 2).

Following the phlebogram a needle was placed into a vessel and the limb was raised so as to empty the vessels. A tourniquet was applied above the ankle and 2 ml of 1% aethoxysclerol was injected into the vessel. The tourniquet was released after 5 min. The procedure was repeated 6 times at 8 hourly intervals.

A phlebogram carried out 1 month later showed disappearance of the dilated vessels seen prior to treatment (Fig. 3).

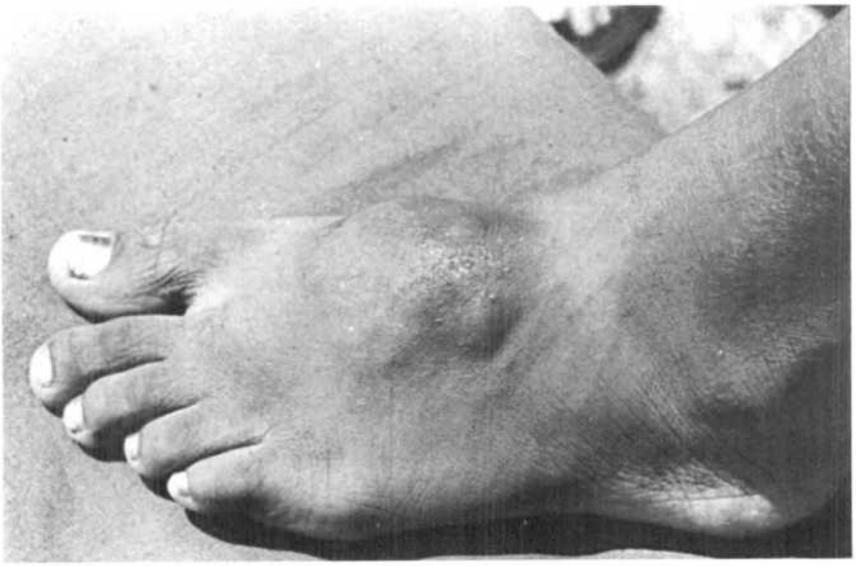

FIGURE 1. Sudoriparous angioma on the dorsum of the left foot.

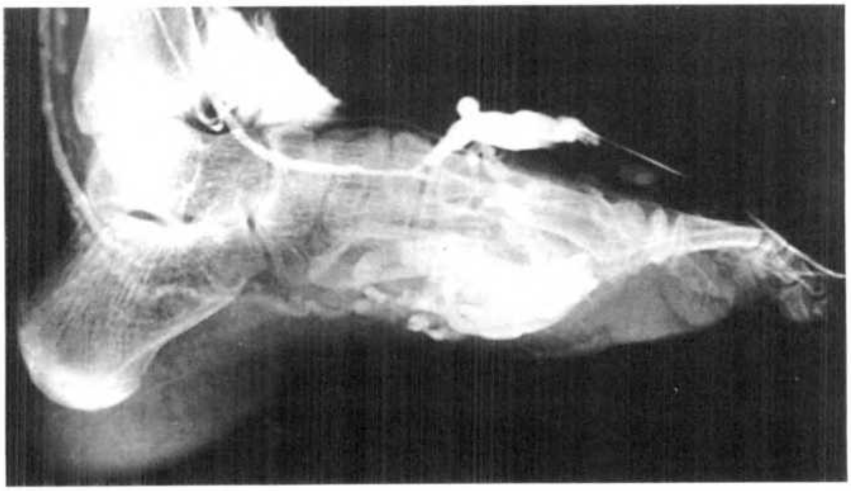

FIGURE 2. Phlebogram before injection of sclerosing agent.

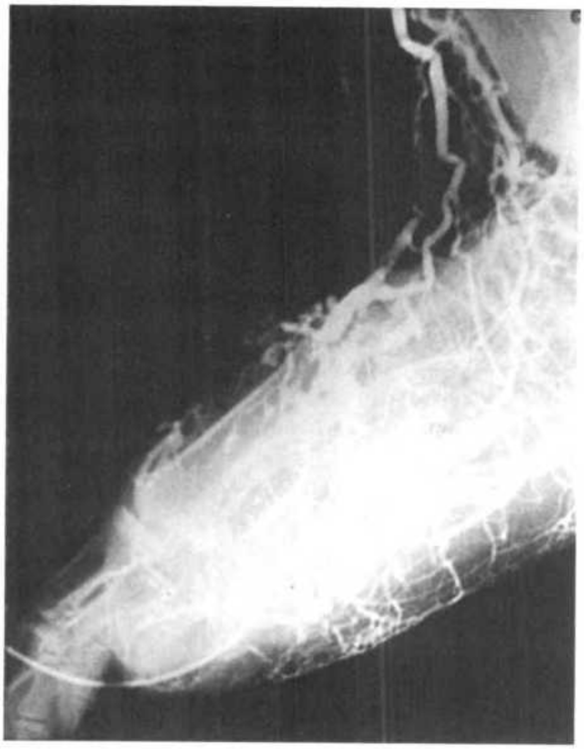

FIGURE 3. Phlebogram 1 month after injection of sclerosing agent.

DISCUSSION

Blue rubber bleb naevus, in which hyperhidrosis is known to occur,[5] was initially considered as a possible diagnosis but ruled out as there was no gastrointestinal involvement. Biopsy was not attempted because of the possibility of uncontrollable bleeding.

The authors are of the opinion that injection of a sclerosing agent should be considered as a therapeutic measure in selected cases of sudoriparous angioma.

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Giant cell arteritis and systemic lupus erythematosus

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SUMMARY

We describe a case of histologically proven giant cell arteritis (GCA) in a 62-year-old woman who initially presented with upper limb girdle pain and weakness, and intermittent claudication. Clinical improvement was obtained with oral prednisolone.

She subsequently developed a photosensitive rash and was diagnosed as having systemic lupus erythematosus.

The features of the two conditions and the possibility of an association are discussed.

CASE REPORT

The patient, a 62-year-old woman, was well until several weeks after fracturing the left tibia in October 1985 when she developed pain and stiffness in the shoulders and hips. This responded rapidly to a short course of Voltarol. In February 1986 she experienced an episode of extreme pain in the shoulders and arms on drying herself after a hot shower. This pain was relieved by resting the arms. At about the same time she became aware of difficulty in getting out of the bath and remarked that, because of upper limb fatigue, she found she was only able to do one row of knitting at a time. A few weeks later, in April 1986, when she was able to get out of doors, she noticed pain in both calves after about 200 yards of walking and she was referred to a vascular surgeon who, in May 1986, noted that the pulses in both limbs were impalpable and arranged for elective investigation. In June 1986, she had a recurrence of episodes of painful, cold-related, colour changes in her hands, characteristic of Raynaud's phenomenon, a condition which she had had in her youth. On review, in July 1986, she had a haemoglobin level of 9·3 g/dl and an ESR of 130 mm/h. This was repeated and the haemoglobin found to be 9·6 g/dl and the ESR 52 mm/h. At no time did she complain of headache, visual disturbance, difficulty or pain on chewing, scalp tenderness, weight loss or fever. Her only medication had been Voltarol. She had smoked five cigarettes daily until 1980. The only family history of note was that her mother had died of a myocardial infarction at the age of 58 years.

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On admission to hospital she was pale, with absent brachial, radial, popliteal, posterior tibial and dorsalis pedis pulses. The blood pressure could not be measured. There were bilateral carotid and femoral bruits. The temporal arteries were palpable and not tender. The upper limb pulses were impalpable and she was found to have developed livedo reticularis and foci of cutaneous ischaemia on her lower limbs. Visual acuity was not impaired.

Investigations revealed an improving haemoglobin of 11·6 g/dl with a dimorphic picture and an ESR of 26 mm/h. The white cell count, platelets, urea, creatinine, electrolytes, calcium, phosphate, glucose, total protein, liver function, thyroid function tests, creatine kinase, protein electrophoresis, cryoglobulins, syphilis serology, hepatitis B serology, urinary microscopy, urinary protein (including Bence-Jones protein), creatinine clearance, lung function tests, ECG, chest X-ray and computerized tomography of the head, were all normal or negative. Autoantibodies were negative except for thyroglobulin at a titre of 1:640 and thyroid microsomes at a titre of 1:40; IgM anticardiolipin antibodies were weakly positive (the lupus anticoagulant was absent); cold agglutinins were present at a titre of 1:8 to adult cells only at 4°C, but negative to adult and cord cells at room temperature and 37°C. In a biopsy of normal, light protected skin, definite granular IgM deposits in the basement membrane zone were evident on direct immunofluorescence. Doppler studies of the lower limbs suggested superficial femoral artery narrowing. An aortogram showed a normal aorta and common and external iliac arteries, but the superficial femoral arteries were narrowed. Digital subtraction angiography revealed narrow carotid arteries, but normal renal arteries. The brachial arteries were not visualized. These findings were reported as being consistent with a widespread arteritis. An arterial biopsy was considered to be indicated and a temporal artery biopsy was performed. The intima was thickened and cellular, there was fragmentation of the internal elastic lamina and a moderate chronic inflammatory cell infiltrate was seen in the media and intima; the lumen was narrowed. The histological findings were considered to be those of giant cell arteritis (GCA).

Therapy was promptly instituted with prednisolone at an initial dose of 60 mg daily. The patient improved symptomatically and the carotid bruits disappeared, although the peripheral pulses remained absent. She was discharged feeling better with no claudication, a normal haemoglobin of 13·1 g/dl and a normal ESR of 2 mm/h. The dose of prednisolone was gradually reduced. In January 1987 she reported low back pain and X-rays showed collapse of the second and fourth lumbar vertebrae. Azathioprine and oestrogen replacement with Premarin were introduced. Shortly after this, in February 1987, she contracted herpes zoster in the L5-S1 distribution. During a sunny holiday in Portugal in May 1987, whilst taking prednisolone 5 mg o.d., azathioprine 50 mg t.d.s. and Premarin 0·625 mg o.d., she developed a symmetrical eruption of red, scaly, raised patches on the dorsal surfaces of the fingers, the radial knuckles of the hands and on the forearms (Fig. 1). She was otherwise well, but on examination of the cardiovascular system the carotid bruits were found to have returned. A peripheral white cell count revealed a leukopaenia of $2{\cdot}9 \times 10^9$/l with a normal differential count, but the haemoglobin, platelet count, ESR and all other investigations were normal or unchanged. Antinuclear antibodies were negative, although on review the ANF had been temporarily positive at a titre of 1:10, as had the rheumatoid factor at a titre of 1:640, 6 months previously. The lupus anticoagulant was not detected although low levels of IgM anti-cardiolipin antibodies persisted. A skin biopsy showed hyperkeratosis and parakeratosis with a chronic inflammatory infiltrate in the dermis, most marked in a periadnexal distribution. Immunofluorescence of involved skin showed granular deposition of IgM and C_3 at the basement membrane zone. Immunofluorescence of uninvolved, light protected skin, showed IgM at the basement membrane zone. These findings supported a diagnosis of lupus erythematosus. The

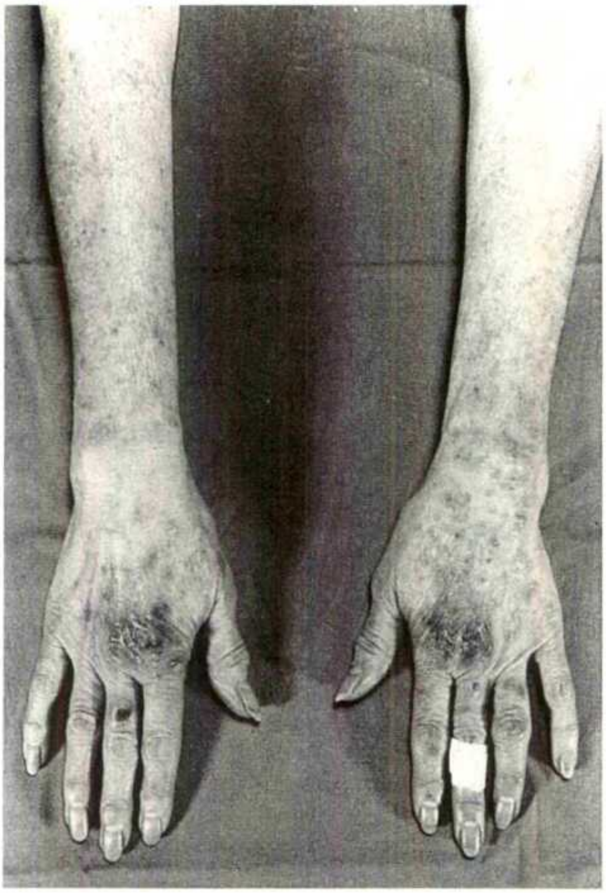

FIGURE 1. Erythematous, scaly, raised patches on the dorsal surfaces of the fingers, the radial knuckles and the forearms.

rash responded poorly to potent and very potent topical steroids and the use of a factor 15 sunscreen, in addition to the systemic therapy listed above. She developed arthralgia in July 1987 and was started on hydroxychloroquine. Her compliance with anti-malarials was poor and her condition remained unchanged.

DISCUSSION

There are several points of interest in this case. Firstly, there is the prominence of the symptoms of intermittent claudication and upper limb ischaemia and the finding of bruits. Claudication was the complaint that prompted her referral from the orthopaedic surgeon who was managing the fractured tibia to the Department of Vascular Surgery. In the series of Healey *et al.*[1] claudication was a presenting feature in only one of 50 patients with a positive temporal artery biopsy; claudication pain in the leg was experienced by only four of the 50 patients at any time during the course of their illness. In the series reported by Jonasson,[2] three of 124 patients with positive biopsies presented with upper limb claudication and two with both upper and lower leg ischaemic pain. Huston[3] reported claudication in one of 42 patients with either a positive temporal artery biopsy or an elevated ESR plus a tetrad of clinical features, before diagnosis and in a further patient after diagnosis. Hamilton *et al.*[4] recorded one case of upper limb and five cases of lower limb claudication in their series of 25 biopsy-positive patients. The series reported by Klein[5] contained 22 patients with upper or lower limb claudication or both out of a total of 248 cases with a positive biopsy, and four of these patients presented with claudication. In all of these reports claudication occurred in 4–24% of patients although it is not noted at all in two other series of 19[6] and 126 patients,[7] respectively. The incidence of bruits has not been widely reported, perhaps because they have not been widely sought. However, Hamrin *et al.*[8] found them to be frequent; where careful auscultation of patients was performed, 30 out of 52 patients were found to have bruits. Angiography was performed in five cases and confirmed arterial stenosis at the sites of bruits.[8] Other reports do not allude specifically to the presence of bruits, but large vessel involvement has been well recognized by other criteria. Klein *et al.*[5] suggested that large artery involvement was common in GCA with 34 of their 248 patients showing evidence of involvement of the aorta or of its major branches. Their patients had intermittent claudication (22), parasthesiae (2), Raynaud's phenomenon (5), transient ischaemic attacks (4), abdominal angina (1), myocardial infarction (1), absent or decreased pulses (not enumerated), and bruits (not enumerated). Angiography was performed in 10 of their patients and indicated that arteritis rather than atherosclerosis was the cause of the large vessel disease. Three patients died of aortic rupture and, at post-mortem, widespread GCA was found.

A second point of interest is the occurrence of Raynaud's phenomenon. Although Raynaud's phenomenon is not generally considered to be a feature of giant cell arteritis, it is mentioned as occurring in five patients in Klein's series of 248 cases.[5] In our patient, however, it appeared to be a recurrence of an old problem and may have been induced by a reduction in hand blood flow as a consequence of upper limb arteritis.

A third point is the behaviour of the ESR and haemoglobin levels (Fig. 2). The ESR fell steadily, despite worsening of the patient's symptoms, from the time of the first estimation in April 1986 to diagnosis in August 1986, and subsided to normal promptly after the initiation of prednisolone therapy. The shape of the curve in Figure 2 suggests that the ESR would have

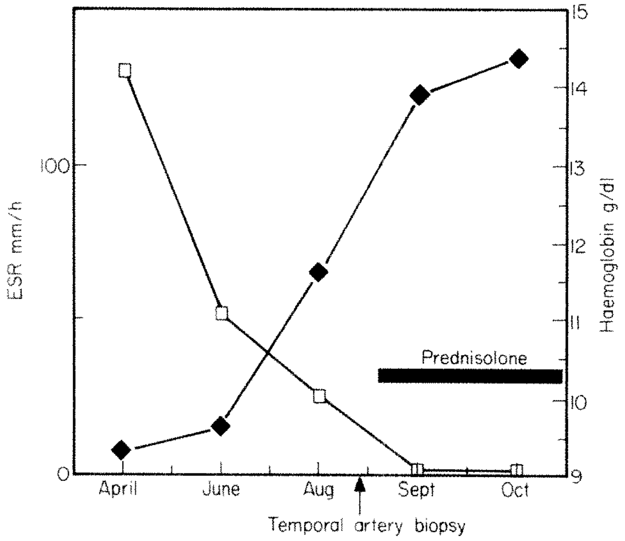

FIGURE 2. Changes in the ESR (□) and haemoglobin level (◆).

become normal spontaneously and independently of steroid treatment. That this sequence of events may be occurring in those patients with giant cell arteritis whose ESR is normal at the time of presentation (18 out of 80 patients in one series)[9] is worth consideration. Our patient's haemoglobin level behaved inversely to the ESR and, by interpolation of the curve, might also have been normal at the time that steroid therapy was instituted. To the best of our knowledge this phenomenon has not been reported previously.

Finally, there is in this case the association of giant cell arteritis and lupus erythematosus. The presence of livedo reticularis, an IgM band at the dermo-epidermal junction of normal skin, cold agglutinins and equivocal anticardiolipin antibodies suggested the possibility of latent, or evolving, LE, despite absent ANF and ENA, Ro and La antibodies. The subsequent development of a photosensitive discoid rash, arthralgia and a leukopaenia confirmed this eventuality; enough of the American Rheumatism Association criteria[10] were present to make a diagnosis of SLE. Giant cell arteritis has not been reported to occur in any particular association with lupus erythematosus although periarteritis nodosa was reported as a concurrent disease in one of Jonasson's 124 patients.[2] A large vessel intracranial arteritis with giant cells has been reported in one case of SLE.[11] Large vessel arteritis is rare in SLE, the characteristic feature in central nervous system LE is small vessel vasculopathy with hyalinization and fibrinoid necrosis.[12] It is interesting that our patient had positive immunofluorescence of uninvolved, light protected skin at presentation. Immunoglobulin deposits in the skin have not been found before in GCA. In a study which included immunofluorescence findings, none of 10 patients with GCA had positive immunofluorescence nor did 25 controls; however, most patients with SLE (14 out of 17) and half those with rheumatoid arthritis (six out of 12) had positive immunofluoresence.[13] Livedo reticularis and anticardiolipin antibodies have recently been subjects of interest in SLE. Livedo reticularis may have other causes than SLE and is a normal variant in young women, but it has not been previously associated with GCA.[14] Anticardiolipin antibodies are present in approximately 40–60% of patients with SLE[15] and have recently been associated with livedo reticularis in SLE,[16] in addition to the previously recognized association with central nervous system disease and thrombotic events such as spontaneous abortion[15] and more recently with renal disease and vasculitis.[16] Livedo reticularis and central nervous system involvement (Sneddon's syndrome) is a known association, but only recently has livedo reticularis been linked with renal disease and vasculitis in SLE and proposed as a cutaneous marker for the later development of important systemic events in SLE.[16]

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British Journal of Dermatology (1988) **119,** 121–125.

Perioral blisters in a bug-biting baby

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SUMMARY

We report peri-oral blistering caused by biting a bug, *Palomena prasina*, commonly found in the U.K. This is just one of a number of insects commonly found in the U.K. which produce chemicals for defensive purposes that may injure human skin. Contact with insects should, therefore, be considered as a cause of blistering eruptions, even in Britain, especially in the young who are more likely to handle insects.

Insect bites commonly cause blisters, but dermatologists rarely consider the possibility that a blister could be due to the human biting the insect.

We report a 6-month-old child who developed blistering around the mouth after biting a bug which is common in all parts of the British Isles.

CASE REPORT

A 6-month-old boy presented in June 1986 with erosions around the mouth. The previous day he had been on the lawn in the garden when he suddenly began crying. Next to him, his mother found a crushed green 'beetle' which had a pungent smell, and which she presumed he had tried to chew. Over the next few hours, he developed erosions around the mouth and lips (Fig. 1), which healed over the subsequent few days, without scarring. The 'beetle' was identified as *Palomena prasina*, more commonly known as the green shield or 'stink' bug, due to the pungent exudate released as a defence against handling.

A similar bug was, subsequently, crushed and applied to the forearm of one of the authors, producing an irritant reaction at the site of application. They are also said to taste 'acrid' if eaten (J.H.Martin personal communication).

DISCUSSION

Arthropods produce several defensive secretions which range from specialized exocrine secretions, to blood or enteric discharges which contain toxic compounds. The Spanish fly

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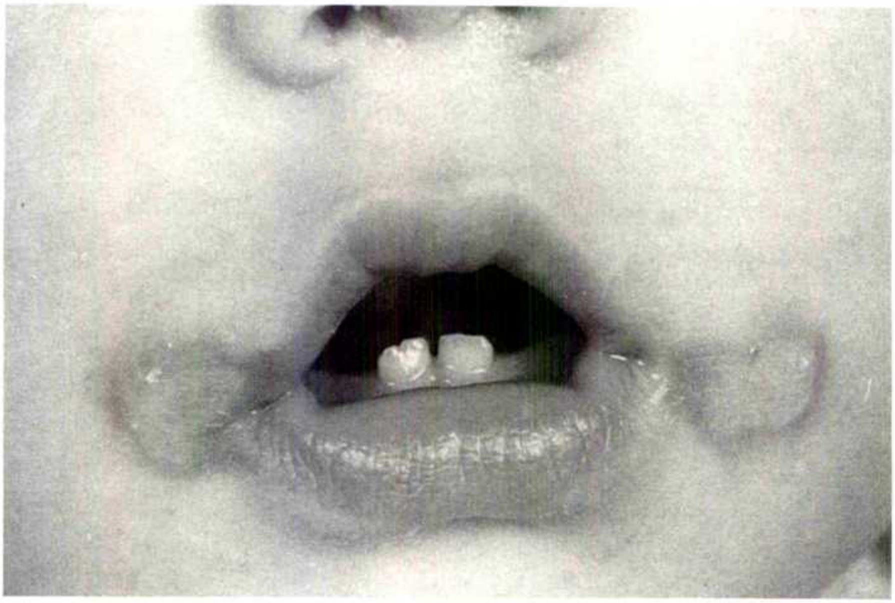

FIGURE 1. Mouth of infant several hours after biting the bug *Palomena prasina* showing perioral blistering.

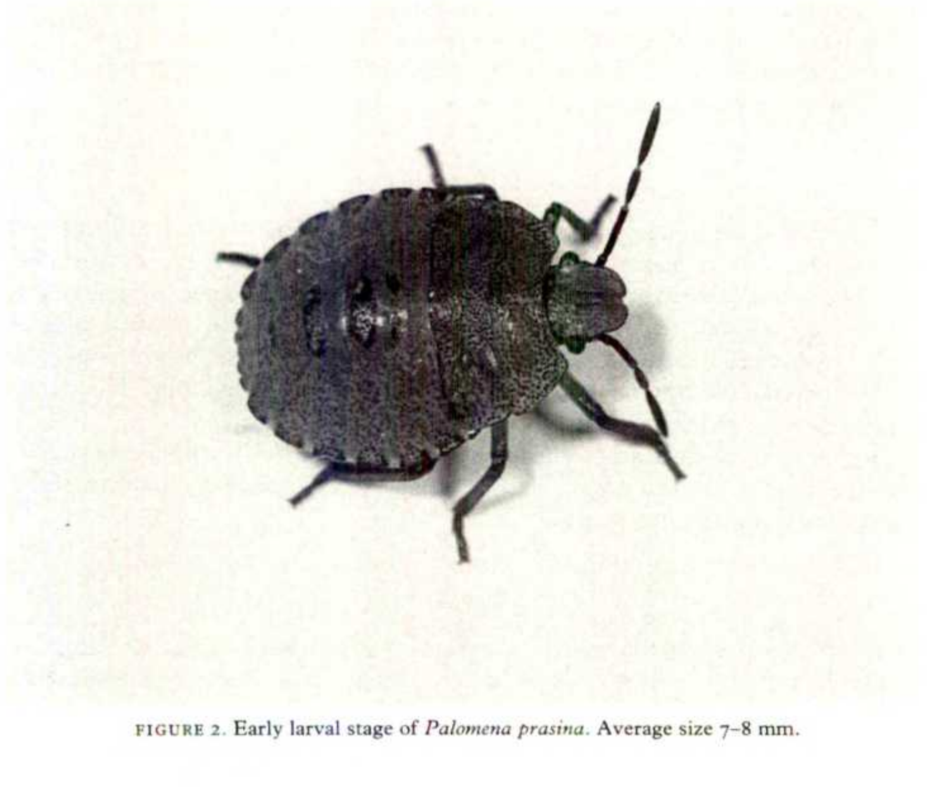

FIGURE 2. Early larval stage of *Palomena prasina*. Average size 7–8 mm.

(*Lytta vesicatoria*), for instance, produces a terpenoid anhydride[1]—cantharidin—present in blood and tissue, which is a vesicant and has been used to treat warts. Similarly, certain members of the Staphylinidae (Rove beetles), in the genus *Paederus*, are responsible for blistering eruptions in Africa, South America and Australia, having blood enriched with a toxin called pederin.[2,3]

Palomena prasina (the green shield bug) belongs to the family Pentatomidae (order Hemiptera) and along with the other true bugs described below is commonly found in Britain during the summer months.[4] It occurs in meadows, the margins of heaths, woodland clearings and parks. The new generation is found from September onwards and the bright green larvae (Fig. 2) darken during November (Fig. 3), so that by hibernation they are a dark bronze-red colour. The adults (Fig. 4), emerge in May and are bright green; mating and egg laying occur in late June and July. During this time, they are found on many deciduous trees and shrubs; hazel being a common habitat. They are also frequently found in domestic gardens especially on beans.[4] The European relative of this bug, *Palomena virridisima* is known to produce a number of defensive chemicals[5] which are also likely to be present in the British species (B.W.Staddon, personal communication). These comprise the reactive aldehydes trans-2-hexanal, trans-2-octanal, trans-2-decanal (Fig. 5) and tridecane (a C-13 alkane) which facilitates the penetration of the aldehydes through the cuticle of the insect. These compounds fulfill their defensive role by means of their irritant nature and unpleasant odour and they might be expected to prove irritant to human skin with which they come into contact. Some other shield bugs have also been shown to produce ketoaldehydes such as trans-4-oxo-hex-2-anal (B.W.Staddon, personal communication) which, being more polar, are more likely to be irritant than the corresponding

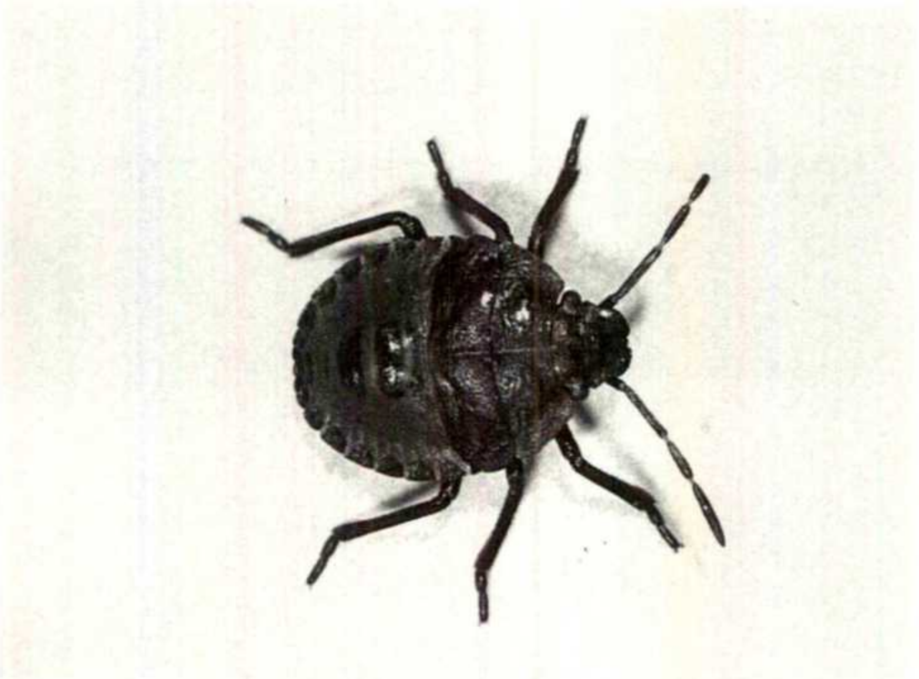

FIGURE 3. Late larval stage of *Palomena prasina*. Average size 7–8 mm.

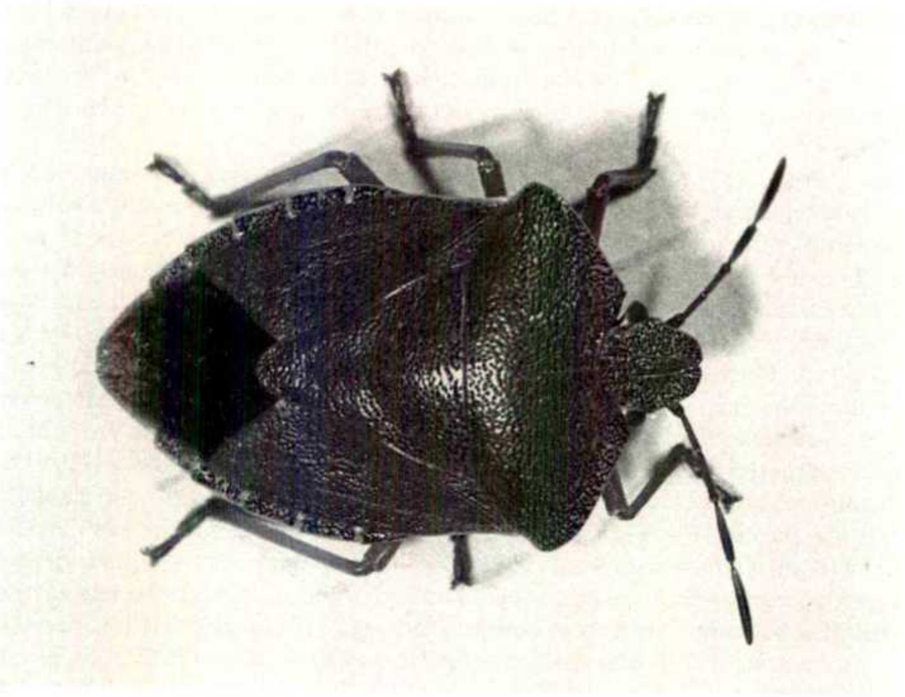

FIGURE 4. Adult *Palmonena prasina*. Average size 1·5 cm.

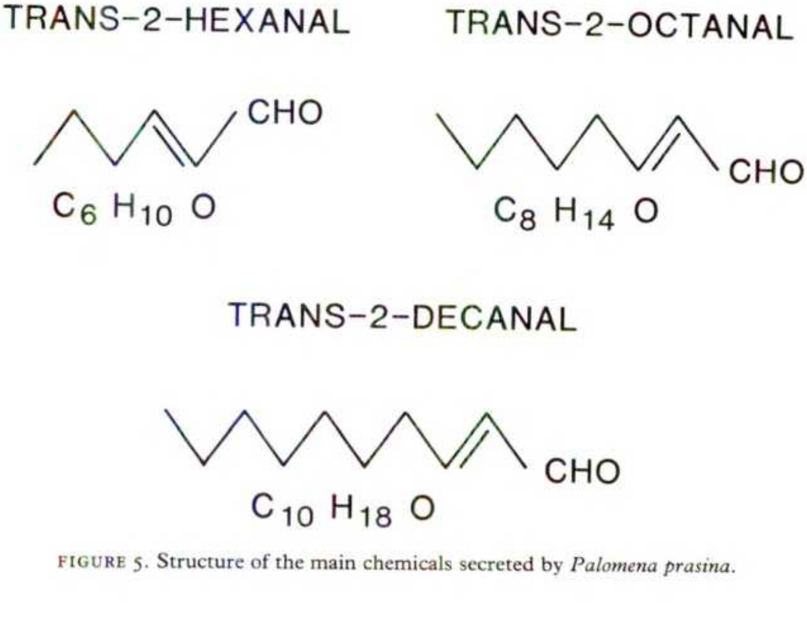

FIGURE 5. Structure of the main chemicals secreted by *Palomena prasina*.

aldehydes (via the carbonyl group forming Schiff bases). These latter compounds have not, however, been identified in *Palomena prasina*.

Several other classes of insects which might come into contact with man in the British Isles also produce defensive chemicals. For instance, some beetles (Coleoptera), and ants (Hymenoptera) produce hydrocarbons, aldehydes, ketones and esters, which may have defensive functions. These exert their effect on predators in different ways, such as by altering the generation characteristics of antennal chemoreceptors, rendering them temporarily anosmic (eg. hydrocarbons[6]), by damaging chemoreceptor proteins (eg. conjugated aldehydes[5]) and by creating 'white noise' due to non-specific chemoreceptor stimulation by mixtures of chemicals.[5] An example of the latter are the 44 volatile compounds secreted by the ant *Camponotus herculeanus*[7] which allow it to escape predators under the cover of its defensive 'chemical smokescreen'. Another mechanism is employed by the harvestman beetle which produces secretions fortified with chemicals including 4-methyl-3-hepatanone[8] and the cockroach (*Platyzosteria armata*) which produces 2-hepatone as a constituent of its defensive spray.[9] Both of these are alarm pheromones of ants, causing them to exhibit temporary panic behaviour, giving the arthropods time to escape these formidable predators. Yet a third mechanism of defence is by the production of a range of chemicals which by means of their unpleasant odour and irritant nature make the bugs unattractive to predators. This mechanism is exemplified by two families of the true bugs (Heteroptera)—the Acanthosomidae (eg. the hawthorn, juniper and birch shieldbugs) and the Pentatomidae (eg. the green, gorse and green vegetable shield bugs), the latter group containing the bug responsible for the blistering in our patient.

Beetles, ants and bugs are commonly found throughout the British Isles and several of them may prove irritant to human skin. We feel, therefore, that contact with insects should be considered as a cause of blistering eruptions even in Britain, especially in young children, who are more likely to handle the insects.

ACKNOWLEDGMENTS

The authors would like to extend their thanks to Dr B.W.Staddon, Department of Zoology, University College, Cardiff, for help regarding the secretions of *Palomena prasina* and Dr J.H.Martin, Department of Entomology, British Museum, Cromwell Rd., London, for help in the identification of larvae and information regarding the consequences of biting this bug.

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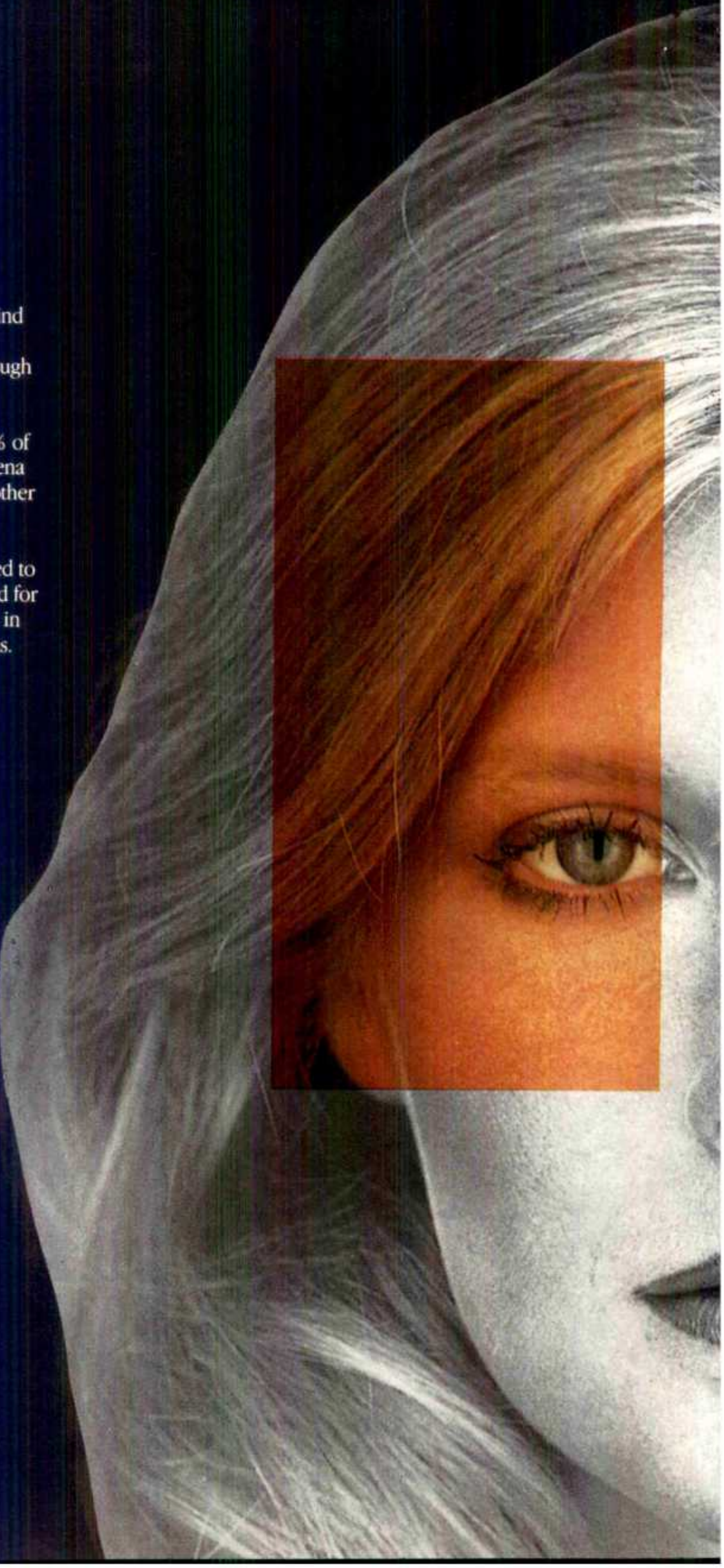

British Journal of Dermatology (1988) **119,** 127–134

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Lupus vulgaris simulating a port-wine stain

MADAM, Lupus vulgaris can simulate the clinical appearance of a port-wine stain very closely. However, any confusion between the two conditions can be easily resolved by a detailed history, as lupus vulgaris is not present at birth, while a port-wine stain is usually present at birth and with increasing age there is a colour shift from pink to purple. We would like to report a patient who had lupus vulgaris which closely simulated a port-wine stain.

A female patient, aged 67 years, was referred by a consultant plastic surgeon for an opinion about the possibility of treating a port-wine stain with laser therapy. The patient reported that she was born with apparently a small birthmark on the lobe of one ear which was cauterized on two occasions and treated with superficial X-rays in childhood. There was no other history of trauma. Subsequently, the lesion seem to spread onto her face, particularly over the last 10 years (Fig. 1). There was no other significant past medical history and no family history of tuberculosis. The patient had applied small amounts of topical corticosteroid to the lesion from time to time. On examination, the patient had a vascular-looking violaceous eruption involving most of the right side of her face. Biopsy confirmed the clinical diagnosis of

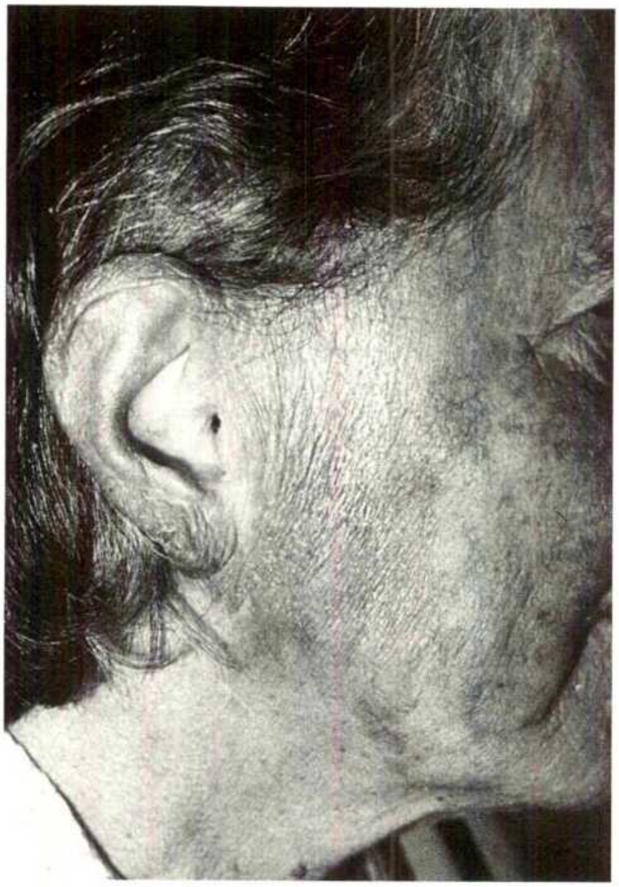

FIGURE 1. Appearance of the patient's right cheek.

lupus vulgaris and the condition responded very well to combination therapy with rifampicin and isoniazid.

The lupus vulgaris in this patient simulated a port-wine stain so closely that she was referred by a consultant colleague for consideration of laser therapy. From the history it is likely that the patient was born with a small port-wine stain involving the earlobe. The main clue to the diagnosis came from the history, which detailed considerable extension in recent years, which would be very unusual for a port-wine stain. Port-wine stains may occasionally be acquired, but this is a very rare event.[1]

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Plasma insulin response to oral glycaemic stimulus in lichen planus

MADAM, An increased incidence of carbohydrate intolerance,[1-3] similar to that seen in type II diabetes mellitus,[3,4] has been observed in patients with lichen planus. An abnormality in the insulin response to oral glucose challenge together with the absence of demonstrable islet-cell antibody has also been demonstrated in these patients.[3] The insulinogenic index, the ratio relating enhancement of circulating insulin to the magnitude of the corresponding glycaemic stimulus, is more helpful than absolute values in interpreting plasma insulin concentration.[5]

In an extended study of glucose tolerance in lichen planus,[4] we studied the plasma insulin response to oral glycaemic stimulus in 56 randomly selected patients (aged 6–70 years) with untreated, idiopathic and histologically proven lichen planus. Venous blood was collected at time 0 (fasting), and 30, 60, 90 and 120 min after 75 g (1·75 g/kg ideal body weight) oral glucose, for the estimation of blood glucose and plasma insulin levels, during the standard oral glucose tolerance test (OGTT). The blood glucose level was estimated by the method of Asatoor and King[6] and the plasma insulin concentration was estimated by using a modified double antibody technique.[7] The criteria for abnormality of glucose tolerance were those suggested by the National Diabetic Data Group (1979).[8] The insulin/glucose ratio was calculated by dividing the insulin concentration (μU/ml) by the corresponding blood glucose level (mg/dl).

The results of the standard OGTT showed that of the 56 patients, 39 (70%) had normal glucose tolerance, 15 (27%) had impaired glucose tolerance and two (4%) were overtly diabetic. The mean fasting plasma insulin levels were almost identical in patients with normal glucose tolerance, than those with glucose intolerance and those with overt diabetes. After the glucose load, however, in patients with normal glucose tolerance, an approximately 4·5-fold increase in insulin level was found after 30 min while this increase was threefold or less in patients with glucose intolerance. The mean plasma insulin levels at 120 min in the OGTT were higher in patients with overt diabetes and significantly higher in patients with impaired glucose tolerance ($P < 0{\cdot}05$) compared with patients with normal glucose tolerance (Table 1), thus showing a delayed and more prolonged insulin response in these two groups and suggesting a degree of insulin resistance.

Hence, there was a delayed and more prolonged hyperinsulinaemia together with hyperglycaemia in lichen planus patients with glucose intolerance, as is seen in mild maturity onset diabetes mellitus where the critical functional defect in early diabetic beta cells lies in the speed of response to a rising blood glucose level rather than the total quantity of insulin secreted relative to normal beta cells.[9] When circulating insulin levels after glucose loading were evaluated in the context of the glycaemic stimulus the patients with impaired glucose tolerance and overt diabetes had lower values than patients with normal glucose

TABLE 1. Mean plasma insulin levels (μU/ml) in lichen planus patients with normal glucose tolerance, impaired glucose tolerance and overt diabetes mellitus. Values are means ± SD

		Time (min)				
Glucose tolerance	No. of patients	0	30	60	90	120
Normal	39	7·68 ± 2·61	33·94 ± 15·02	40·41 ± 27·19	32·17 ± 19·80	23·95 ± 15·12
Impaired	15	8·13 ± 7·08	28·27 ± 15·40	46·30 ± 21·30	41·66 ± 16·32	40·50 ± 16·78*
Overt diabetes	2	8·40 ± 1·30	20·80 ± 2·90	26·50 ± 3·50	34·20 ± 3·10	38·00 ± 5·90

*Significantly higher than corresponding value in patients with normal glucose tolerance, $P < 0{\cdot}05$, Students' *t*-test.

tolerance, similar to that seen in type II diabetes, indicating lower insulin levels relative to the degree of hyperglycaemia in these patients.

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Need for specialist follow-up of patients with basal cell carcinoma

MADAM, We would like to comment on statements made in the discussion of the article on therapy of basal cell carcinoma (*British Journal of Dermatology* 1987, **117**, 349). Dr Harrison recommends the use of shave excision and cautery as a primary method of treatment, *with subsequent follow-up by the referring general practitioner* (our italics). The rationale is that 'recurrences . . . are likely to be negligible'. He goes on to say 'The patient is given instructions concerning care of the wound and asked to report to their general practitioner if there are any problems or recurrence. This saves inconvenience . . . and saves health care resources.' We believe remarks such as these overlook an important appreciated factor about the subsequent course of patients with basal cell carcinoma (BCC).

It is recognized that individuals with a history of BCC will regularly develop more skin cancers. Epstein[1] found that 20% of 139 patients given a follow-up appointment 1 year after a BCC was treated had developed new BCCs; and of these new lesions 75% were unsuspected by the patient. More recently

Robinson[2] reported that 36% of her patients with BCC developed one or more additional BCCs within 5 years. On Kauai we have followed prospectively all non-melanoma skin cancer (NMSC) patients for 5 years. New skin cancers were detected in over 30% of these unselected individuals (unpublished data). Although most new lesions were BCCs, squamous cell carcinomas were regularly observed, and melanomas were occasionally encountered.

When the dermatologist does the follow-up, new cutaneous malignancies are regularly discovered at much earlier stages of development in patients with a history of BCC. In our practice, lesions found at follow-up examination are commonly less than 6 mm in diameter. Such lesions are amenable to simple out-patient procedures which might, in the long run, 'save health care resources'.

Dr Harrison also gives the impression that follow-up is not necessary 'in an ageing population with significant natural mortality'. The articles by Epstein[1] and Robinson[2] suggest the opposite.

We have no quarrel with Dr Harrison's method of treating BCC and accept that in his experience recurrences are unlikely. However, your readers should be aware that patients with a history of NMSC are at significant risk for the development of new skin cancers. For this reason, we feel these patients deserve careful follow-up by physicians who are experts in skin cancer.

We urge our dermatological colleagues to think of themselves as more than just mechanics who fix what is broken. Once we recognize a pathology, then it is our job to aid in preventing the situation from occurring again. With this attitude of 'prophylactic dermatology' new tumours greater than 6 mm in diameter are rarely encountered. Patients appreciate both the interest expressed in their care and the infrequent need for painful procedures and occasional disfigurement which tumours diagnosed late can require.

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Reply

We agree with Drs Burnett and Elpern, that there are other considerations, apart from low recurrence rate, which might dictate the need for follow-up of patients after basal cell carcinoma therapy. The development of new tumours is relevant, but if the patients and the patients' referring general practitioners are given clear instructions to report to the doctor if there are any problems or recurrence (or development of a new tumour) we believe that many repeat visits may be unnecessary, thus saving patient inconvenience and health care resources. Obviously, if a patient's tumour is in a difficult site or the tumour has had an aggressive history, or if a patient has had a tendency to multiple lesions, then follow-up may be necessary. In addition, there are geographical considerations. Patients in our cloudy, rainy area often have only one or two tumours, rarely more, whereas patients in other climates may be prone to multiple tumours (particularly patients with certain skin types) and follow-up for these patients may thus be more necessary.

Data on patients receiving therapy for basal cell carcinoma is stored on computer, which allows us to recall patients at risk, and this information can give a referring general practitioner an indication of the need for follow-up when appropriate. We have analysed the data for 1987 and compared that year with 1986 (figures in brackets). In 1987, 119 patients (77) presented to the Lancaster Dermatology Department for surgical treatment. There were 61 males and 58 females, age range 21–94 years, mean 65·7 years (69·8). There were an additional 12 patients (6) referred for radiotherapy; 38 patients (41) had shave excision, 25 patients (12) had curettage and cautery, 43 patients (15) had straight excision, eight patients (0) had delineated excision and five patients (3) had excision and graft or a wedge excision. Fourteen patients (3) had tumours in extra-facial sites and 16 patients (6) had multifocal, superficial spreading or morphoeic tumours. Only six patients (8) had recurrence of tumours treated. Furthermore, only nine patients had previously received therapy for other tumours, indicating the low incidence of other tumours in our patients (possibly reflecting skin types and low sun exposure in our population). The number of patients receiving shave excision in 1987 was similar to that in 1986. However, there was an increased percentage of patients receiving straight excision and curettage and cautery in 1987 compared with 1986. This reflected both an increased number of superficial friable tumours, but more importantly, an increased number of larger, deeper tumours or tumours in more difficult sites in 1987. Most of these patients were followed up after treatment, however, 48 patients (27) were not followed up after therapy in 1987 and most of these patients—83%—had received therapy utilising curettage and cautery or shave excision for small or less difficult tumours. Furthermore, there was a tendency for more elderly patients not to receive follow-up. Thus, simpler or smaller tumours in more elderly patients, particularly if receiving simple treatment were less likely to be followed up by the clinic.

In summary, we would recommend shave excision as an alternative to curettage and cautery in some patients with simple or smaller tumours, but would re-emphasize that the method is not suitable for more difficult tumours or tumours in difficult sites which should receive other therapy (either by a dermatologist, plastic surgeon or radiotherapist). We would defend our policy of minimal follow-up in some patients, particularly those with simpler tumours, and more elderly patients (important in a centre serving a predominantly rural area with possible transport difficulties). Furthermore, this follow-up policy seems suitable for our population at the present time, given the good liaison with local general practitioners, the low incidence of multiple tumours in the area and non-excessive UV exposure. With increasing leisure exposure to UV in a younger population our policy may have to be modified if tumour patterns alter.

There are obviously going to be individual approaches to treatment, and, indeed, regional and international differences in therapy, which may reflect patient or resource differences. Our approach to therapy, we believe, is flexible and allows for these differences, but also attempts to treat patients as individuals.

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Laser treatment of port-wine stains

MADAM, The study by Adams *et al.* (*British Journal of Dermatology* 1987, **117**, 487), which examined optimal laser parameters for treatment of port-wine stains, was of great interest, and underlines the need for improvements in therapy of vascular lesions. Unfortunately, the paper, as it is written, equates selective absorption of light with selective damage of vessels, by disregarding the importance of exposure duration.

The similarity which these authors observed between responses to 'selective visible light' and 'non-selective near infra-red light' is in fact not surprising in view of the methods used. The pulse duration employed (0·2 s) is substantially greater than the calculated thermal relaxation time for vessels of the size commonly seen in port-wine stains, e.g., approximately 1 ms.[1] Consequently, heat conduction during the laser exposures by both wavelengths causes relatively non-selective damage to the superficial layers of the skin, regardless of the microscopic location of the primary absorber of energy, namely the vascular contents in the case of the argon laser. This has been previously demonstrated by Greenwald *et al.*,[2] who showed uniform necrosis of the superficial layers of the skin following argon laser therapy, in contrast to highly selective damage only, when very short pulses were delivered. Therefore, Adams *et al.* were not actually comparing selective versus non-selective treatment modalities, but rather, two non-selective modalities produced by two different wavelengths.

Selective vascular heating is indeed not a requirement for successful therapy. For example, carbon dioxide laser energy, which is strongly absorbed by water, can be used with benefit for treatment of vascular lesions.[3,4] Nevertheless, selective vascular injury appears to result in less scarring; the potential benefits of the tunable dye laser at 577 nm in this respect were alluded to by Dr Adams. This type of laser exposure, developed from a theoretical model,[1] using a 300 μs pulse duration, has now been applied to a large number of patients with vascular lesions of various types, who have been successfully treated without scarring, and is now our standard treatment for port-wine stains and facial telangiectasia. However, an increased clinical response (lightening of lesions) relative to argon laser therapy has yet to be documented, and the patients who best repond to this type of treatment have still to be defined.

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3 Ratz JL, Bailin PL. The case for use of the carbon dioxide laser in the treatment of port-wine stains. *Arch Dermatol* 1987; **123:** 74–5.
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Reply

Madam, Selectivity is a comparative and not an absolute commodity. There can be little argument that argon laser light is more selectively absorbed by blood than Nd:YAG laser light. For example, more than 95% of argon laser light (488 and 514 nm) will be absorbed in 1 mm of well oxygenated blood, while less than 15% of ND:YAG laser light (1064 nm) will be absorbed by the same amount of blood. While selectivity of absorption of laser light by blood may well differ from selectivity of damage to blood vessels, confining the concept of selectivity to particular pulse lengths or wavelengths while regarding all others as non-selective seems a too constraining definition.

The Wellman group have made an important contribution to the understanding of the pathogenesis of selective laser damage of blood vessels by drawing attention to the process of heat conduction from the laser heated blood vessels into the surrounding tissues. We agree that selectivity of laser damage to small blood vessels may be reduced by using pulse lengths longer than their thermal relaxation time. We hope that their observation of increased selectivity of 300 μs pulses of 577 nm which we mentioned in our discussion will be translated into superior clinical cosmetic results in patients with port-wine stains.

In our comparison of the argon laser with the Nd:YAG laser, we used those parameters of power and pulse duration that are used by the vast majority of groups using lasers to treat port-wine stains. Short pulse lengths of the order of 300 μs could not have been attained with the lasers available to us.

Increase in selectivity of damage to vessels may be expected to reduce the post-treatment epidermal necrolysis and, hopefully, late scarring, but our work stresses that these side-effects are not the most important feature limiting the cosmetic effect. Rather, it is the primary failure of the laser method to produce permanent blanching that seems to us the feature that most limits the final cosmetic result. While we would welcome a reduction in the incidence and severity of side-effects, we feel that future research should also concentrate on increasing the efficacy of methods to achieve permanent blanching of the abnormal vessels in order to produce a better final cosmetic result.

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Bowen's disease and internal malignancy

MADAM, Drs Eedy and Gavin (*British Journal of Dermatology* 1987, **117**, 715) may be correct in their belief that patients with Bowen's disease have an increased incidence of internal malignancy, but the evidence as presented is wanting in several respects. In a retrospective cohort study they report that of 157 patients with Bowen's disease 18 developed internal malignancy, that this was greater than the number expected, and that this difference was statistically significant ($P = 0{\cdot}014$). The P value alone tells us that this result was unlikely to have occurred by chance, but without details of the expected number and the population at risk we are left ignorant of the relative risk and the attributable risk. The former is important in allowing the reader to assess the strength of the association and to allow us to consider whether unknown bias may affect the conclusions, and the latter in the assessment of what value screening may have.[1] Depending on the magnitude of the relative risk, the estimation of the calculated under-registration of internal malignancy in the Northern Ireland Cancer Registry may assume importance. The authors quote a figure of 37% for under-registration. Was this known to be constant during the 13 years of study and what effect would the confidence limits for this estimate have on the size of the relative risk? It may well be that possible bias would increase the size of the effect the authors demonstrate, thereby increasing the significance we should attach to their conclusions.

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REFERENCES

1 MacMahon B, Pugh TF. *Epidemiology: Principles and Methods*. Little, Brown and Co. Boston. 1970.

Reply

MADAM, We welcome this opportunity to deal with the issues raised by Dr Rees and provide additional infomation on our study population. The population of Northern Ireland is 1·56 million and over the 13-year period of our study, 157 cases of Bowen's disease were diagnosed, giving an average of 12 cases per year. In this group of 157 cases the expected number of internal malignancies was 10·0 (7·1 for females and 2·9 for males) compared with the observed number of 18 (11 females and seven males). The relative risk for the study group was 1·8, being 1·6 for females and 2·4 for males. This would seem to represent a high relative risk of an individual with Bowen's disease, subsequently developing an internal malignancy and since the risk is approximately twice that of an individual in the general population, it would seem prudent to screen patients with Bowen's disease for internal malignancy. The attributable risk calculated in terms of person years of risk for the study group was 10 per 1000 person years, being 6·5 per 1000 person years for females and 23 per 1000 person years for males. Clearly with the relatively low incidence of Bowen's disease and an attributable risk of this magnitude it would be totally inappropriate to screen a population for Bowen's disease in an attempt to detect the relatively few cases of internal malignancy which may be associated with Bowen's disease in any general population.

While it is not known whether the under-registration in the Northern Ireland Cancer Registry varied during the 13 years covered by the study, the number of total annual registrations in the registry during the 13 years remained relatively constant at 4500 per year, suggesting little variation in registrations over time. Taking the 95% confidence limits for the under-registration of the cancer registry gives the ranges of the relative risks as 1·6–1·8 for females and 1·3–1·6 for males, thus maintaining the high relative risk for the individual presenting with Bowen's disease.

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Severe cutaneous vasculitis induced by diltiazem

MADAM, We read with much interest the recent report by Lambert *et al.* of acute generalized pustular dermatitis induced by diltiazem (*British Journal of Dermatology* 1988, **118,** 308.). We would like to draw your attention to two cases of severe widespread cutaneous vasculitis induced by diltiazem which we have recently reported.[1] Our patients, who were both female (aged 70 and 57 years, respectively), developed a severe cutaneous vasculitis 8 days after administration of diltiazem. Widespread vasculitic lesions developed over 24 h and rapidly became confluent over large areas. In the second patient there was a marked systemic disturbance and small erosions and pustules were present in some areas. Pustule formation can be a feature of a florid cutaneous vasculitis. In both instances gradual resolution occurred 2–3 weeks after withdrawal of the drug.

Cutaneous side-effects are said to occur in 1% of patients treated with diltiazem and most are considered to be minor.[2] However, more serious adverse reactions, such as exfoliative dermatitis, have been reported.[3] Our experience and that of Lambert *et al.* suggest that severe cutaneous reactions may be more common than has been previously realised. Diltiazem is a relatively new drug and it seems probable that the incidence of severe cutaneous adverse reactions will rise as its use increases.

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REFERENCES

1 Sheehan-Dare RA, Goodfield MJ. Widespread cutaneous vasculitis associated with diltiazem. *Postgrad Med J* (in press).
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3 Larvijsen, APM Van Dijke, C Vermeer B-J. Diltiazem-associated exfoliative dermatitis in a patient with psoriasis. *Acta Dermatovenereol* 1986; **66:** 536–8.

British Journal of Dermatology (1988) **119**, 135–138.

Book Reviews

Transdermal Controlled Systemic Medications. Edited by YIE W.CHEN (1987) New York: Dekker. Pp 464. Price US $95.00.

Most of this volume consists of edited versions of lectures given at a symposium in 1985. It is of interest to pharmacologists and internists more than to dermatologists, but overlaps our interests at various points, particularly for those concerned with percutaneous absorption and related problems. It is disappointing to note (in Chapter 3) how little methods for promoting skin permeability have advanced in the last 20 years.

The main appeal of this volume will be to academic pharmacists and those involved in industry in developing new therapeutic agents for topical use. Particularly valuable will be the final section of the book on regulatory requirements of the various national authorities in the U.S.A., Japan and the U.K.

H.BAKER

Hexachlorobenzene: Proceedings of an International Symposium (International Agency for Research in Cancer, Lyon, France–June 1985). Edited by C.R.MORRIS AND J.R.P.CABRAL (1986). Oxford: Oxford University Press. Pp 691.

The earliest and most dramatic demonstrations of hexachlorobenzene (HCB) toxicity were the outbreaks of HCB-induced hepatic porphyria in Turkey in 1954–59. Subsequently, HCB has been vigorously studied throughout the world and identified as an environmental pollutant and poison of considerable diversity and importance.

Despite prohibition of its commercial production, HCB still accrues from industrial synthesis of other polyhalogenic compounds and there is an abiding interest in it in the contexts of toxicology, medicine, ecology and associated disciplines.

These Proceedings constitute an important collation of reports from an international constellation of experts dedicated to study of the toxic, genotoxic, porphyrinogenic and (possibly) oncogenic properties of HCB, together with papers reporting studies of ecological parameters of persisting pollution.

Contributors to the compilation total almost 400 and their combined papers number 88, classified into seven main sections, each being appropriately subdivided. Subject coverage is surprisingly broad, yet this has not precluded the inclusion of extensive experimental details in several papers nor the useful graphical presentation of results. Porphyria as it relates to HCB is well covered, a number of authors including George Elder and Derek Cripps being well known in the dermatological literature. The style is generally clear and easy to read, but with some variation, of course, from author to author.

This publication will particularly interest toxicologists, hepatologists, specialist chemical pathologists, experimental oncologists and ecologists, but dermatologists interested in the causal association between HCB and porphyria will also find the appropriate sections of the book extremely useful.

D.C.NICHOLSON

Les Eczemas Allergiques. J. FOUSSEREAU (1987). Paris: Masson. Pp 616. Price 580F.

This is a textbook of contact dermatitis. It covers both allergic and irritant contact dermatitis. It should be regarded as a reference book. Like any good reference book it appears to be comprehensive and to have a thorough bibliography.

The index is complete, but could be improved if the most important page number were printed in heavy type (for instance formaldehyde presently refers to five page numbers and colophany to six). Where appropriate, biochemical formulae are published. However, illustrations of plants are not included. Another omission is a table of common allergens listing the substances in which they are frequently found.

Hexamidine-induced allergic contact dermatitis is covered clearly. Hexamidine is a widely used topical antiseptic in France. Revuz *et al.*[1] have described 20 cases of a reaction to hexamidine characterized by papules and vesicles and, histologically, by leukocytoclasis. These reactions last sometimes more than a month and it seems that they may be mediated by an Arthus reaction.

At a price of 580F (approximately £60) and with a text written in French this book will have limited appeal in Britain and other Anglo-Saxon countries. Nevertheless, it is a clear and well written text and should be commended to those with an interest in contact dermatitis and to French speaking dermatologists.

C.M.E.ROWLAND PAYNE

REFERENCE

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Skin Deep. T.A.GROSSBART AND C.SHERMAN (1987). New York: Wm Morrow. Pp 289. Price US$17.95.

This book is written jointly by two doctors of philosophy; Dr Grossbart, who is a clinical psychologist and a member of the Faculty of Harvard Medical School, and Dr Sherman, who has had a career in medical journalism. It is not surprising, therefore, that the book is easy to read. The authors state that the book is aimed at both dermatologists and their patients, and particularly at patients with longstanding dermatological problems where emotional and psychological factors may be playing a part in pathogenesis.

The book is divided into four main sections. The initial chapters are essentially about the development of insight, whilst the second part deals with therapeutic techniques, including biofeedback, psychotherapy and blocking the itch/scratch cycle. The third and fourth sections of the book deal with assessing the results of therapy detailed earlier in the book and finally there is a section on definition of common dermatological terms, such as psoriasis and alopecia, which clearly would be helpful to patients, but largely irrelevant to the dermatologist.

I think this book would appeal to a motivated patient, and one at the more obsessional end of the clinical spectrum. The authors are careful to stress that their approach is to be regarded as an adjunct to conventional dermatological therapy, and there is nothing here to upset conventional dermatological approaches to skin problems. Self-help groups such as the Psoriasis Association and the Eczema Society may be interested in informing their members about this book.

There is clearly more in this book for the patient than for the dermatologist.

J.A.COTTERILL

A Colour Atlas of Allergy. W.F.JACKSON and R.CERIO (1988). London: Wolfe Medical Publications. Pp 126. Price £35.00.

This book is one of a series of medical atlases. It is incorrectly titled. It is an atlas of immunological disorders, not just allergy, covering conditions ranging from Addison's disease and AIDS, to food intolerance and lupus erythematosus. Ophthalmic, otorhinological, dermatological and gastro-enterological aspects are covered. There are also chapters covering mechanisms, investigation, drug allergies, auto-immune diseases and immune deficiency.

The illustrations, which are mainly in colour, are in general of good quality, with a few notable exceptions, e.g. herpes gestationis, Raynaud's phenomenon and dermatomyositis affecting the hands. The text is simple, clear and, I am pleased to say, keeps to mainstream views regarding allergy. There are, however, occasional omissions and errors: the failure to include topical steroids in the management of eczema, pruritus mis-spelt, and the implication that in pemphigoid, large doses of steroids need to be taken for 2–3 years before being reduced.

Seen in isolation, I feel it is a book without a readily identifiable market. I suppose the aim is for it to find its way onto a medical student's or general practitioner's shelf, in combination with the others in the series.

It is an attractively presented basic book, but each subject is not treated in sufficient depth for it to be recommended to the specialist.

M.H.BECK

Kaposi's Sarcoma, Pathophysiology and Clinical Management. Edited by J.L.ZIEGLER and R.F.DORFMAN (1988). New York: Marcel Dekker. Pp 266. Price US$78.

Dermatologists throughout the world have been well acquainted with the peculiar vascular tumour known as Kaposi's sarcoma since the original description by the Hungarian dermatologists of 'idiopathic multiple pigmented sarcoma of the skin' in 1872. For more than 100 years since the lesion was first described, Kaposi's sarcoma has been the subject of extensive investigation and controversy. This timely monograph is an excellent and comprehensive review of the current state of knowledge. The book is multi-author and most of the chapters are clearly and informatively written with good illustrations and little overlap of subject matter. Aspects of the tumour considered in this volume include the pathology, histogenesis and aetiology of the tumour as well as the clinical features and staging and a discussion of the various treatment modalities available. One of the best chapters is the first chapter by the editors giving an up-to-date overview of the history, epidemiology, clinical and pathological features. This is followed by a competent review of the pathology of Kaposi's sarcoma by Dr Templeton which quite rightly stresses the difficulty in diagnosis in the early stages of the disease and discusses the pathological differential diagnosis. The widespread distribution of lesions throughout the body is also emphasized.

The chapter on histogenesis by Dorfman, Wood and Beckstead is also excellent and while these contributors are careful to give all the available evidence a fair treatment, they do suggest that there is good evidence that Kaposi's sarcoma, at least in a proportion of cases, is probably derived from lymphatic endothelium. The role of cytomegalovirus in the aetiology is discussed in Chapter 4. A large amount of evidence linking cytomegalovirus infection to Kaposi's sarcoma exists, but evidence for a causative role still remains somewhat circumstantial.

Paul Volberding contributes a very useful and well illustrated chapter on the clinical features and staging of the condition. His comment on the histopathological variation, dividing Kaposi's sarcoma into three variants does seem somewhat arbitrary and although the clinical staging system proposed by workers at New York University is the most widely used, because of the rather special nature of Kaposi's sarcoma and the variation in clinical course between patients, the usefulness of such a staging system is rather controversial.

Susan Wall contributes another chapter which is likely to be most useful for the practising clinician, discussing the radiological features of Kaposi's sarcoma. There are, in particular, helpful comments on the chest X-ray diagnosis of pulmonary Kaposi's sarcoma, a real problem in diagnosis for clinicians and radiologists alike.

The two final chapters in the book discuss the management of Kaposi's sarcoma with radiation therapy and chemotherapy respectively. The authors of these two chapters are based in California and probably have more experience in treating Kaposi's sarcoma of the AIDS associated variety than most other workers around the world.

Dr Ziegler and Professor Dorfman have put together in this volume an excellent and comprehensive review of this enigmatic vascular tumour. The authors suggest that the volume may be of interest to oncologists, AIDS physicians, pathologists, radiotherapists, dermatologists and immunologists. This is undoubtedly the case and one can think of workers in many other specialities who will find this text invaluable. This book is undoubtedly the best single monograph on the subject available today.

N.P.SMITH

Books Received

1987 Year Book of Dermatology. Edited by A.J.SOBER and T.B.FITZPATRICK (1987). Chicago: Year Book Medical Publishers. Pp 498.

Clinical Immunodermatology. M.V.DAHL (1988). 2nd edition. Chicago: Year Book Medical Publishers. Pp. 422.

British Journal of Dermatology (1988) **119**, 139–140.

News and Notices

Fellowship of the Royal College of Physicians of London

The following members of the BAD have recently been elected to the Fellowship of the Royal College of Physicians of London:

Dr Paul Jeffrey August
Dr Michael John Cheesbrough
Dr Jocelyn Roger Corbett
Dr Paul Albert Dufton
Dr Angus Leith MacMillan
Dr Andrew Charles Pembroke
Dr John Darrel Wilkinson

Third Cosmetic Dermatologic Surgery Seminar

The Third Cosmetic Dermatologic Surgery Seminar will be held from 2–6 November 1988, co-sponsored by Northwestern University and the University of Miami. The seminar will cover the topics of ageing skin, laser surgery, chemical peel, liposuction and soft tissue augmentation. Breakfast roundtables with faculty. The Registry Hotel on the beach at Naples, Florida.

Further details from: J.B.Pinski, M.D. 55 E. Washington St., # 3404 Chicago, IL 60602, U.S.A.

Seventh Postgraduate Course in Medical Mycology (Dermatomycology)

The seventh Postgraduate Course and Workshop in Medical Mycology will be held on the Campus of The University of California School of Medicine, San Francisco, U.S.A. from 14–16 October 1988. The course is designed for dermatologists, clinical pathologists, mycologists, pediatricians, nurses, medical technologists, clinical microbiologists, and other paramedical personnel. Lectures will emphasize clinical, immunological, pathological, and epidemiological aspects of the various mycoses. An AIDS update will be included. Laboratory sessions, supervised by the faculty, will stress self-assessment and simplified approaches to office dermatology. All presentations will be illustrated and accompanied by cultural and microscopic demonstrations.

A special guest faculty joins the faculty at UCSF to provide this programme. Much of the information presented will be geared to those preparing for specialty boards, but the material will also be useful to those desiring to broaden their concepts of infectious diseases.

For further information contact: Extended Programs in Medical Education, Room 569-U, University of California, San Francisco, CA 94143, U.S.A. Telephone (415) 476-4251.

Arnold Rikli Prize

For investigations in the field of photobiology in relation to human beings we are awarding the Arnold Rikli Prize amounting to SFr.20,000.—. This prize is sponsored by the Institute Freidrich Wolff AG, Riehen/Basel (Switzerland) as a regular establishment. It will be awarded annually beginning in 1989.

The reports to be presented should cover investigations on the biopositive systemic effects of optical irradiation (100–3,000 nm) on human beings. They should be of direct significance in practical application or provide guidelines for future research.

The reports must be written in German or English and reach our office by 31 December 1988. An international jury of experts will evaluate the documents to determine the winners; no appeals will be considered.

The Arnold Rikli Prize will be awarded for the first time in 1989.

For further information, please contact: Institut Friedrich Wolff AG, Störklingasse 44, CH-4125 Riehen/Basel, Switzerland.

International Conference on Behçet's Disease

An International Conference on Behçet's Disease will be held at the Mayo Clinic, Rochester, Minnesota, U.S.A. on 14–15 September 1989. For further information contact: Mr William Nietz, Continuing Education, Mayo Clinic, Rochester, Minnesota 55905, U.S.A.

Erratum

The importance of the coal tar bath in the Ingram treatment of psoriasis. Evaluation by evaporimetry and laser Dopler flowmetry. Frödin *et al.* 1988; **118:** 429. The last sentence of the discussion should read: However, a recent study has shown that this combination reduces the UVB dose by a mere 10% of the UVB dose in combination with the vehicle only.

British Journal of Dermatology (1988) **119,** 141–145.

Review

The role of intercellular adhesion molecules in inflammatory skin reactions

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Accepted for publication 10 March 1988

A newly discovered disease involving the inability of leukocytes to mediate adhesion related functions has recently been described.[1-5] The molecular basis of this disease called leukocyte adhesion deficiency (LAD) is a defect in the gene coding for the common beta chain of the lymphocyte function associated antigen-1 (LFA-1) family of adhesion molecules.[6-9]

Clinical manifestations of LAD include recurrent and progressive soft tissue infections, diminished pus formation, impaired wound healing, granulocytosis, and delayed umbilical cord separation.[1-5] The frequency and severity of the infections appear to be directly related to the amount of adhesion molecules expressed on the patient's leukocytes. Thus severely deficient patients who express less than 0·3% of the normal amount of adhesion molecules on their granulocytes have many more infections than moderately deficient patients whose leukocytes express up to 31% of the LFA-1 family of adhesion molecules.[10]

In vitro, the role of the LFA-1 family of adhesion molecules has been well characterized. Antibodies to LFA-1 inhibit lymphocyte functions requiring cell–cell contact.[11] They inhibit mitogen responses, plaque-forming cell responses and cytotoxic activities[12-15] by preventing lymphocyte–accessory cell or lymphocyte–target cell interactions.[16-18] Furthermore, lymphocytes from LAD patients display the same defective reactions *in vitro* as do their normal counterparts in the presence of anti-LFA-1 antibodies.[19-20]

Granulocyte functions inhibited by antibodies to Mac-1 (the granulocyte adhesion molecule) or to the common beta chain, include aggregation, binding to C3bi coated particles, chemotaxis in agar[21] and adherence to endothelium.[22,23] Again, granulocytes from LAD patients display the same defective properties as do their normal counterparts in the presence of antibodies.[1-5,24] Thus, the clinical symptoms seen in LAD patients are attributed to a defect in the ability of their leukocytes to adhere to cellular and extracellular substrates. For example, leukocytes are unable to migrate to sites of inflammation in these patients due to their inability to attach to endothelial cells at the inflamed location. Furthermore, lymphocyte defects are also apparent in these patients since the bulk of their antibodies are produced against T-independent antigens due to the lack of cell–cell interactions required for presentation of T-dependent antigens.

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INTERCELLULAR ADHESION MOLECULE-1

The finding of LFA-1 dependent, antigen independent homotypic adhesion of an Epstein-Barr virus (EBV) transformed B lymphoblastoid cell line and a rapid means of detecting this adhesion made it possible to identify an epitope of LFA-1. Based on the observations that, first, the EBV transformed B cell line JY aggregated in the presence of phorbol esters and that this aggregation was inhibited by anti-LFA-1 antibody, secondly, that EBV transformed B cells from LAD patients that do not express LFA-1 do not form homotypic aggregates and, thirdly, that EBV transformed B cells from LAD patients do form aggregates with JY cells; it was concluded that EBV transformed B cells from LAD patients express a ligand of LFA-1 which is capable of binding to LFA-1 on the JY cells.[25] This ligand was identified by monoclonal antibody RR1/1 which was raised from spleen cells of mice immunized with EBV transformed B cells from LAD patients.[26] The molecule identified by RR1/1 was called intercellular adhesion molecule-1 (ICAM-1) and was found on a variety of lymphoid and non-lymphoid tissue using immunohistological and immunofluorescence techniques. ICAM-1 was expressed on vascular endothelial cells, thymic epithelial cells, some haematopoietic cells such as tissue macrophages (Fig. 1), mitogen-stimulated T lymphocytes, EBV transformed B cells, and differentiated U937 and HL-60 cells.[27] Perhaps more importantly, ICAM-1 expression was more pronounced at

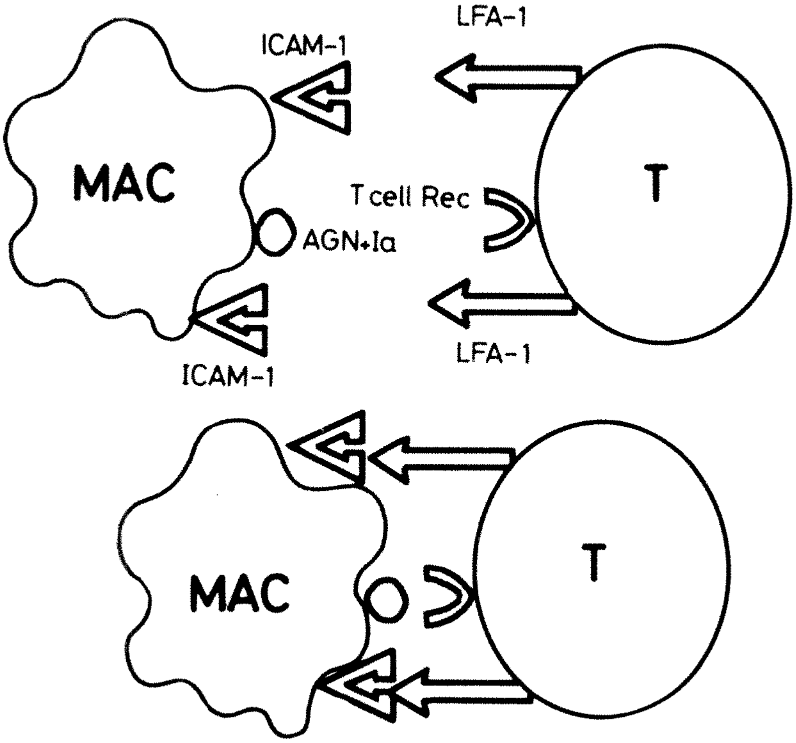

FIGURE 1. A representation of cell adhesion involving LFA-1 and its ligand ICAM-1. In this example a macrophage (MAC) is presenting the antigen (AGN) to a T cell.

inflammatory sites and was induced *in vitro* on fibroblasts and endothelial cells by inflammatory mediators such as interleukin-1, tumour necrosis factor and gamma interferon.[27-29]

Biochemical characterization of ICAM-1 shows it to be a glycoprotein with a molecular weight ranging from about 90 to 115 kd depending on the cell type from which it was immunoprecipitated. Its expression can be inhibited by the protein synthesis inhibitors cyclohexamide and actinomycin D.[27]

Functionally, antibodies to ICAM-1 inhibit homotypic lymphocyte adhesion as well as lymphocyte adhesion to fibroblasts, endothelial cells and ICAM-1 incorporated into planar membranes in the same manner in which anti-LFA-1 antibodies inhibit this adhesion, except that the antibodies to ICAM-1 act on the fibroblast or endothelial cell while the antibody to LFA-1 acts on the lymphocyte.[26,27,30]

Thus, ICAM-1 is an inducible protein that appears at inflammatory lesions and mediates lymphocyte–accessory cell adhesion and may play a critical role in immune reactions.

ICAM-1 EXPRESSION IN INFLAMMATORY SKIN REACTIONS

The expression of ICAM-1 in cryostat sections of biopsies from various skin lesions was revealed using a three stage immunoperoxidase method or by the APAAP technique.[31] It was found that ICAM-1 was expressed on keratinocytes from biopsies of benign cutaneous skin lesions such as allergic contact eczema, lichen planus, pemphigoid or pemphigus, exanthema and urticaria. In lichen planus there were keratinocytes that stained more intensely for ICAM-1 at sites with heavy mononuclear cell infiltration, suggesting that ICAM-1 may play a role in mediating this infiltration. Scattered ICAM-1 was also observed in the dermal infiltrates in these lesions. The expression of ICAM-1 on keratinocytes from skin biopsies of patients with exanthema and urticaria was less pronounced, which corresponded with a less pronounced dermal infiltrate. Endothelial cells in biopsies from benign skin lesions expressed ICAM-1 to a greater extent than endothelial cells from normal skin biopsies. No ICAM-1 was expressed on the cell surface of keratinocytes or the very few mononuclear cells in normal skin.

Unlike lesions from benign cutaneous conditions, ICAM-1 expression was less frequently observed on keratinocytes from biopsies of cutaneous T cell lymphoma lesions. Furthermore, there was a tendency for those ICAM-1 positive keratinocytes to lose their ICAM-1 expression in the more advanced stages of the disease.

On the other hand, keratinocytes in all biopsies of lesions taken 48 h after the application of hapten-containing patches to sensitive individuals, expressed ICAM-1 much like the keratinocytes from lesions of benign cutaneous conditions. This expression occurred concurrently with a heavy mononuclear cell infiltrate in the dermis and maximum clinical manifestations. Expression of class II antigens on keratinocytes under these conditions was much less frequent.

When biopsies from irritant contact reactions were studied, in only one of 14 cases keratinocytes expressed ICAM-1 at 48 h, the time of maximum clinical manifestations. This suggests a differential regulation of ICAM-1 in the specific immune response in allergic reactions and the non-specific inflammatory response in irritant contact reactions.

It thus appears that ICAM-1 plays a role in the specific immune response. What is not clear is whether the expression of ICAM-1 on keratinocytes plays a role in initiating the inflammatory process by allowing lymphocytes to infiltrate into the dermis or whether the mononuclear cell infiltrate is producing cytokines which, in turn, induce ICAM-1 on the keratinocytes. The former possibility is feasible since the attachment of lymphocytes to other cell types is an essential step in cell migration, and LFA-1/ICAM-1 interactions mediate the adhesion of lymphocytes to cellular substrates. Thus, it is reasonable that the expression of ICAM-1 on local keratinocytes could provide a matrix onto which LFA-1 bearing lymphocytes can attach and migrate. Alternatively it is possible that the mononuclear cell infiltrate secretes cytokines which induce ICAM-1 on the local keratinocytes since it is known that certain cytokines will induce ICAM-1 on endothelial cells and fibroblasts *in vitro*.[27-29] Thus, it may be that the role of ICAM-1 is not to allow cell migration to inflammatory sites, but rather to facilitate the lymphocyte antigen presenting cell adhesion required in antigen presenting systems. There was also an association between the severity of a lesion and the co-expression of ICAM-1 and HLA-DR. Again, this may be due to a quantitative difference in the amounts of T cell lymphokines present at the lesion. For instance, gamma interferon, a lymphokine known to induce ICAM-1 and HLA-DR on other cell types,[27,28,32] may be locally secreted by T cells in the mononuclear cell infiltrate. What is of particular interest is that more keratinocytes express ICAM-1 than HLA-DR in benign lesions. Since ICAM-1 appears to be expressed on those cell types that have antigen presenting capabilities,[27] it is possible that ICAM-1 is an early marker of antigen presenting activity (Fig. 1). While the role of leukocyte adhesion molecules and their ligands in inflammatory lesions of the skin is just beginning to be studied, it is clear that modulation of their expression or activity will have therapeutic value in the treatment of cutaneous diseases.

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Identification of CD4+, 2H4+(T8γ+) suppressor-inducer cells in normal human epidermis and superficial dermis

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SUMMARY

Immunohistological staining of frozen sections of normal human skin demonstrated the presence of significant numbers of mononuclear cells expressing novel epitopes associated with CD4-positive suppressor-inducer functions. The cells were located around superficial vessels and within the basal layers of the epidermis and hair follicles. The antigen identified by the various antibodies has been shown to be functionally important in the induction of various suppressor cells capable of abrogating B cell responses to pokeweed mitogen. The presence in the skin of cells with possible down-regulatory functions in the immune response may be significant with respect to surveillance against neoplasms and control of appropriate responses to infectious agents.

The skin is a unique immunological environment consisting of a variety of immunocytes, and regulatory cytokines produced by these cells and by keratinocytes. The involvement of Langerhans cells (LC) in ensuring an appropriate immune response to the ingress of various intruders from the environment has been well documented in recent years.[1,2] Both *in vivo* and *in vitro*, it has been shown clearly that such cells are essential antigen-presenting cells for a variety of genetically-restricted T cell-associated immune phenomena. The interactions between haptens or antigens and the HLA-DR-expressing LCs, together with interleukin 1-like molecules such as ETAF[3] explain a variety of clinical observations. These include contact hypersensitivity reactions to simple chemicals, delayed hypersensitivity reactions to microbial antigens expressed by bacteria, fungi and viruses, and there may also be involvement in allograft reactivity and immune surveillance.[4,5] Such reactions involving the two-signal hypothesis of antigen presenting cell activity may be viewed as the positive or 'up-regulatory' side of the immune response. On the negative or 'down-regulatory' side the possible cells of interest are still subject to debate. In this respect the results achieved with murine skin have not been mirrored by the results in humans.

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Two seemingly distinct cell types have been proposed as the counterbalance to LCs in mouse epidermis. Thy 1+ dendritic cells with phenotypic markers associated with NK cells, i.e. T.200, Asialo GM-1, have been shown to be capable of the induction of suppressor T cells in situations where antigens gain access to the body through epidermis depleted of functional LCs.[6,7] Recent studies with T cell receptor probing have indicated that such cells are in all probability immature T cells.[8] The search for a human equivalent of the Thy 1+ cell has been frustrating, as indicated by the absence of positive results using either a variety of antisera against human Thy-1-equivalent molecules,[8,10] or attempts to delineate the presence of T cells in the epidermis.[11] These findings have in fact cast some doubt on the general concept of skin associated lymphoid tissue (SALT) as proposed by Streilein.[12] Using current techniques very few T lymphocytes have been demonstrated in normal epidermis.[13] The second potential down-regulatory cell in mouse skin is the I-J+ UV-resistant cell described in functional assays by Granstein.[14] The relationship of this cell to the Thy-1+ve cell is still in doubt but present evidence indicates that it is likely to be a distinct cell. Again, this is a cell involved in the induction of suppressor T cells. The importance of such cells with respect to a variety of phenomena associated with modulation of skin immunoreactivity after UVB irradiation and with respect to immune surveillance against skin neoplasms have been suggested.[15] No human equivalent of the I-J molecule(s) has been identified and there has been, in fact, no direct localization by immunostaining of the cell in the epidermis. Early studies of Ia antigen in mice included anti-I-J staining controls which failed to demonstrate their presence.[16] This has been a general problem with I-J alloantisera. Our own attempts to achieve localization with monoclonal antibodies have also been discouraging. Increasing accessibility of the I-J epitopes to reagents by neuraminidase digestion may prove to be a solution of this problem.[17]

The increasing availability of monoclonal antibodies to T cell subsets presents opportunities to clarify some of these issues. We report here the observation of significant numbers of cells in normal epidermis and especially around the superficial vascular channels, that express functionally important antigenic moieties associated with the induction of suppression. The older subdivision of T lymphocytes into CD4+T helper/inducer cells and CD8+T suppressor-cytotoxic cells, by antibodies such as OKT4, Leu 3a and b and OKT8, and Leu 2a, has now been expanded. Monoclonal antibodies are now available which define subsets with either helper activity for presentation of antigen to positive (4B4)[18] and negative or suppressive (2H4)[19] Leu 18, WR-16[20] and T8γ (Poulter, personal communication) arms of the immune response. Other antibodies such as Leu 8[21] and D-12[22] have been shown to delineate antigens associated with the suppressor cells and some delineate cytotoxic cells i.e. 9·3.[23] The association of S-100β with cells of the CD-8 lineage, lacking cytotoxic, but possessing suppressive activities has also been shown as an additional marker.[24,25]

METHODS

Normal human skin obtained either from breast reduction operations or from suction blister roofs acquired by the Kiistala technique from volunteers[26] were processed for subsequent immunostaining.

Frozen sections were obtained from material frozen in isopentane-liquid nitrogen, after embedding in OCT, while other samples were freeze-dried and vacuum embedded in paraffin. The latter method[27] has been shown to produce excellent morphology while permitting identification of antigens with monoclonal antibodies which are known to be sensitive to

TABLE 1. Antibodies used in phenotyping skin lymphocytes

Antibody	Source**	Dilution	Cells identified
2H4*	Coulter	1:20	TSI
WR-16	Dr K. Moore[20]	1:20	TSI
Leu 18	Becton-Dickinson	1:20	TSI
T8γ	Dr L. Poulter	1:160	TSI
UCHL-1	Dako	1:160	Pan T reagent
Leu 3a (CD4)	Becton-Dickinson	1:20	T helper-inducer
Leu 2a (CD8)	Becton-Dickinson	1:20	T suppressor-cytotoxic
p25	Dr E. W. Ades[30]	1:160	Human Thy-1 equivalent molecule(s)
4B4* (CD4)	Coulter	1:160	T helper-inducer
T-6 (CD1)	Dako	1:160	Thymocytes/LCs

* Phycoerythrin labelled: Intensity of staining in fluorescence system was insufficient for direct frozen section or freeze-dried section visualization. The antibodies were, however, utilized with the ABC-peroxidase system without difficulty.

Coulter Immunology, Hialeah, Florida, U.S.A.
Becton Dickinson, Mississauga, Ontario, Canada
Dako, Dimension Labs Inc., Mississauga, Ontario, Canada

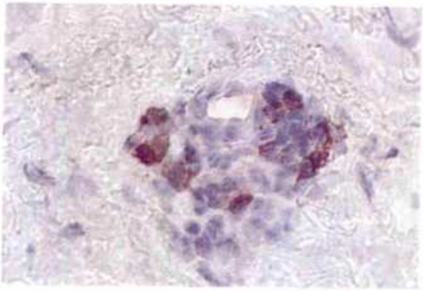

FIGURE 1. TSI staining with anti-T8γ. There are numerous membrane positive cells around a superficial vessel. (ABC-peroxidase, original × 900).

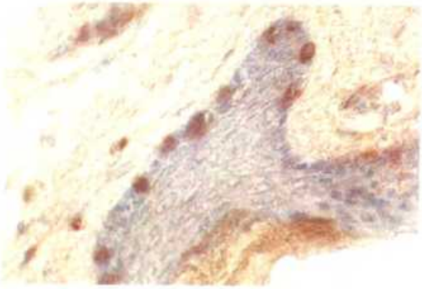

FIGURE 2. TSI staining with anti-T8γ. There are numerous adendritic cells in the basal layer of the epidermis. (Freeze-dried specimen, ABC peroxidase method, original × 400).

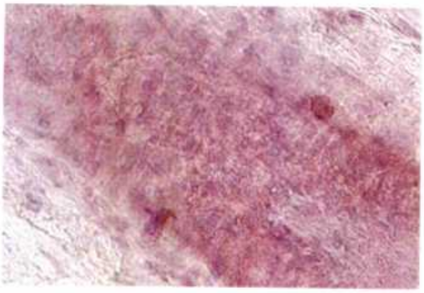

FIGURE 3. TSI staining with 2H4. Dendritic cells can be seen within the outer root sheath of a hair follicle. (ABC peroxidase, original × 900).

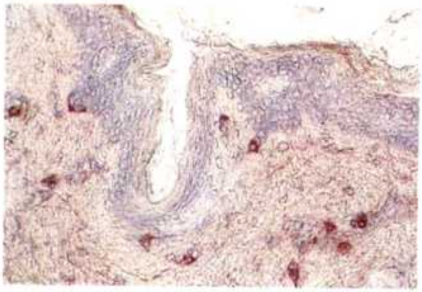

FIGURE 4. Pan-T lymphocyte staining with UCHL-1. Numerous cells are stained around superficial vessels and occasional basal epidermal elements. (ABC peroxidase, original × 200).

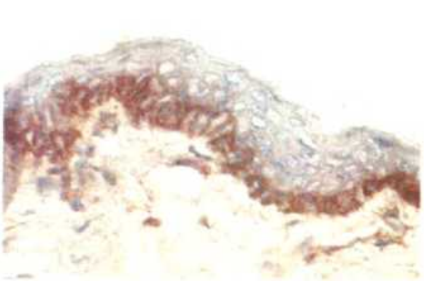

FIGURE 5. Staining with 4B4 showing staining of cells in the superficial dermis and uniform staining of the basal layer of the epidermis. (Freeze dried specimen, ABC peroxidase, original × 400).

aldehyde fixation.[28,29] This material is particularly valuable in that it is permanent and allows later comparisons to be made with a battery of monoclonal antibodies or with newer reagents.

Staining was carried out with a sensitive three step biotin-avidin system employing the Vector ABC peroxidase kit. The final antibody localization was achieved by the standard aminoethyl carbazole (AEC) cytochemical reaction. Sections were briefly counterstained with Mayer's haematoxylin and mounted in Aquamount. The primary antibodies used are shown in Table 1. In addition, a rabbit antiserum against a well defined human Thy-1 equivalent molecule[30] was applied with second step goat anti-rabbit biotin reagents. The controls included omission of primary antibodies, substitution of inappropriate monoclonal antibodies of similar isotypes, omission of second and third step reagents and staining for endogenous peroxidase activity by means of the AEC chromogen reaction.

RESULTS

Twenty-five specimens were stained and the results showed remarkable uniformity, with a clear staining of various T cells in the superficial dermis and in the epidermis and hair follicles.

These can be summarized as follows. The reagents against antigenic moieties associated with T suppressor-inducer (TSI) cells all showed significant numbers of small round cells near the superficial vessels and within the lower layers of the epidermis and hair follicles (Figs 1–3). The numbers of epidermal TSI + ve cells varied, but was approximately one-tenth of the numbers of CD1 + ve LCs seen in adjacent serial sections.

Pan T reagents such as UCHL-1 (which identified formalin-resistant epitopes) gave results which were a composite of those achieved with TSI reagents and those for CD4 and CD8 (Fig. 4). The staining for CD4 (Leu 3a and b) and CD8 (Leu 2a) was less intense and it was clear that many of the TSI cells were probably CD4 + ve. Epidermal CD4 + ve cells were noted with dendritic morphology, but few CD8 + ve cells were seen in the normal epidermis.

The Thy-1 reagent failed to show the presence of the antigen in the samples. 4B4 gave positive staining for many lymphocytes around superficial vessels, but a uniform staining of basal layer keratinocytes was an unexpected observation (Fig. 5). Titration of all reagents showed the immunophenotyping to be accurate and reliable. All controls were negative. In all cases, the freeze dried material gave similar results and showed superior morphology.

DISCUSSION

Morimoto[18,19] has developed two monoclonal antibodies, 4B4 and 2H4, which delineate functionally important subpopulations in the CD4+ group of T lymphocytes. A CD4+ population identified by 4B4 provides excellent help signals for pokeweed mitogen (PWM)-induced B cell differentiation and immunoglobulin synthesis.[18] Such cells proliferate poorly with concanavalin A (Con A) or in autologous mixed leukocyte reactions (AMLR). They are involved with reactions to soluble antigens. 2H4, on the other hand, identifies a population of cells which are poor helpers in the PWM assay, and show high proliferative levels with Con A and in AMLR. The latter cases are known to be associated with the generation of T suppressor cells. It is again present on CD4+ cells. Finally, it has been clearly demonstrated that 2H4 identified a surface antigen of functional significance in the induction of CD8+ suppressor cells.[31,32] The antibody has been used *in vitro* to block the generation of this subset. One recent report of staining with these reagents indicates some differences from the results described here. While most of the cells in the dermis were CD4+, very few epidermal cells were noted with either this reagent or with 2H4.[33] Bos,[34] in his description of the 'skin immune system', (SIS) claims that very few, if any, lymphocytes are present in the normal epidermis, as has been commented on by other authors.[11,13] Some suggestions for these divergent results may be offered. The authors admit in their publication that the immunostaining methods used were of rather low sensitivity, since attempts to double-stain different antigens was the main thrust of the investigation. Failure to demonstrate the presence of CD4 on epidermal LCs due to this low threshold of sensitivity, may also explain why the staining of TSI cells by 2H4 was also not impressive. The paper in fact contains no supporting photographs and shows a diagram summarizing the immunohistology. The results achieved in the present study were consistent and a significant population of TSI cells was always evident in the superficial dermis and to a lesser extent in the epidermis. Of the various TSI reagents the T 8γ reagent proved to be much stronger (by a factor of 10 in titration) than the others. Whether they all identify the same epitopes on the antigen(s) is at present uncertain; indeed, the exact nature of antigen identified by 2H4 is still under investigation. WR-16, for example, appears to identify a CD4+ population involved in the induction of T suppressor cells, but the functional assays indicate that the CD4+ WR-16+ cell does not require the simultaneous presence of CD8+ cells in the assays.[20] This has not been shown to be the case with 2H4 where CD8+ cells are obligatory in the induction of functional suppressor circuits.[19,31,32] Clearly, the suppressor cell circuits are complex, involving a variety of interacting cells and related secreted factors and auxillary cells. Subsequent studies may resolve some of the uncertainties.

A final unexpected observation was the uniform staining of the basal layer of the epidermis with the reagent 4B4. This again was not mentioned by Bos.[33] The reagent clearly stains CD4+ cells both in the skin and in control or tonsil preparations. Whether this is an example of cross-reactivity of no functional significance or is another indication of shared antigens between T cells and keratinocytes remains unanswered. In view of the many indications of the affinity of T cells and keratinocytes (in both normal distribution of thymic factors and epidermotropism in malignant T cell lymphomas,[35,36]), perhaps the expression of the antigens identified by 4B4 has a functional role in the proposed maturation of T cells in adults.

Bearing in mind that various modalities that alter the antigen presenting activity of LCs in the skin, such as UVB, glucocorticoids and tape-stripping have been shown to favour the induction of tolerance, the presence in human skin of cells with supressor-inducer phenotypes is significant. Clearly, studies are needed to define further the numbers and nature of such cells in the face of the above modifiers of immune reactivity. Double staining, FACS analysis and immuno-electron microscopy of cells, with functional assays of suppressor activity in both normal skin and after UVB irradiation should clarify whether the TSI cells reported in the present study are responsible for down-regulatory activities.

Current concepts of skin immunity suggest that the end result of an interaction with hapten or antigen at this environmental interface, is a result of the balance between positive influences mediated via epidermal LCs and possible related dermal cells, and the down-regulatory responses from a balancing suppressor-inducer antigen-presenting cell. Transient or permanent depression of the positive arm of the cutaneous immune response resulting from the interactions of LCs and dermal macrophages with agents such as UVB or corticosteroids, may favour the induction of tolerance. Difference in sensitivity to biological response-modifier cells involved in presenting antigens to the suppressive arms of the immune response, may be of great importance in infectious diseases involving the skin, as well as in immune surveillance against developing neoplasms. Further investigations of the functional capabilities of suppressor-inducer cells in the skin are clearly an important priority.

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Recombinant gamma interferon and *in vivo* induction of HLA-DR antigens

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SUMMARY

Recombinant human IFN-γ, used for treatment of melanoma and renal carcinoma, was found to induce HLA-DR expression on human keratinocytes *in vivo*. HLA-DR antigens bound to keratinocytes of the basal and suprabasal layers of the epidermis were observed after intramuscular or intravenous injections of 0·5 mg/kg body weight IFN-γ, 3 times a week. Keratinocyte-bound HLA-DR antigens were first observed at the beginning of the third or fourth week of treatment, but HLA-DQ and HLA-DP antigens were never detected on keratinocytes. The intracytoplasmic constant (γ) chain of the class II molecules was also not detectable within the keratinocytes. Patients who received IFN-α_2 therapy, did not exhibit keratinocyte-bound HLA-DR antigens.

In normal human epidermis, class II alloantigen expression is restricted to the immunocompetent dendritic Langerhans cells (LC)[1,2] and Sontheimer *et al.*[3] recently demonstrated that all three class II molecules, HLA-DR, -DQ and -DP, are simultaneously expressed by this dendritic cell population. Induction of HLA-DR antigens on keratinocytes has also been reported in diseased skin[4,5] and there is evidence that keratinocytes synthesize these antigens.[6] A common feature of the diseases investigated was a more or less dense cellular infiltrate composed mainly of activated T cells in the papillary dermis. Among other cytokines these cells release interferon-gamma (IFN-γ) and there is evidence *in vitro* that IFN-γ is the principal active substance responsible for the induction of class II alloantigen expression by a variety of cells.[7-12]

An enhanced and selective induction of I-A antigens after *in vivo* administration of IFN-γ has been described in various murine tissues;[13-15] and in the human model strong HLA-DR expression by keratinocytes has been observed after the intradermal injection of IFN-γ in lepromatous leprosy.[16]

It is possible that the cellular expression of class II antigen determinants *in vivo* may reflect a response not only to locally produced, but also to systemically released IFN-γ. We, therefore,

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investigated whether the systemic administration of IFN-γ in humans led to aberrant HLA-DR expression at sites distant from the application site.

METHODS

Patients

Nine patients (six males, three females) with either metastatic melanoma (two males, three females) or metastatic renal carcinoma (four males) were treated. Five patients were treated with rIFN-γ (Genentech, San Francisco, CA, U.S.A., supplied by Bender, Vienna, Austria) over a 1- to 12-week period. One patient with renal carcinoma received three i.m. injections per week and the melanoma patients three intravenous infusions per week. The doses were 0·5 mg/kg body weight. Three additional patients with metastatic renal carcinoma and one patient with metastatic melanoma were treated with IFN-α_2 (Hoffmann-La Roche, Nutley, NJ, U.S.A.) over a 1- to 12-week period. The doses were 18×10^6 IU/day i.m. in the melanoma patient and 5×10^6 IU/day subcutaneously in the renal carcinoma patients.

Skin biopsies

Punch biopsies were taken from the lateral trunk or from the inner side of the upper arm before IFN treatment, and 1 week, 4–6 weeks and 12 weeks or more after initiation of IFN treatment. Samples were snap frozen in isopentane cooled in liquid nitrogen and processed by using a standard three-step immunoperoxidase technique with monoclonal antibodies anti-HLA-DR[17] (diluted 1:300) and anti-HLA-DQ[18] (diluted 1:120), anti-HLA-DP[19] diluted (1:120), and VIC-Y1[20] (diluted 1:600).

Immunoperoxidase technique

Cryostat sections 3 μm thick were thawed and air dried at room temperature. After fixation in acetone for 10 min, followed by fixation in chloroform for 10 min, sections were covered with the monoclonal antibody diluted in Tris buffer containing 1% bovine serum albumin and incubated for 45 min in a moist chamber at room temperature. After three washes in Tris buffer, sections were incubated for 30 min at room temperature with peroxidase-labelled rabbit anti-mouse immunoglobulin (Dakopatts, Copenhagen, Denmark), diluted 1:20 in 50% heat-inactivated human AB-serum (Seromed, Biochrom AG, FRG) in the presence of glucose and glucose oxidase (Sigma, St Louis, MO, U.S.A.). After three washes in Tris buffer, sections were incubated for 30 min at room temperature with peroxidase-conjugated swine anti-rabbit immunoglobulin (Dakopatts, Copenhagen, Denmark), diluted 1:20 in 50% heat-inactivated human AB-serum. After three washes in Tris buffer, peroxidase was developed with 3,3′-diamino-benzidine (Fluka, Buchs, Switzerland) in the presence of H_2O_2. The sections were counterstained with Meyer's haemalum and mounted in Kaiser's glycerine gelatine.

RESULTS

The results are shown in Table 1.

Clinically, three IFN-γ-treated melanoma patients (Patients 1, 3 and 4) showed tumour progression, whereas one IFN-γ-treated patient (Patient 2) and one IFN-α_2-treated patient (Patient 6) remained stable and did not show further tumour growth. All four renal carcinoma patients (Patients 5, 7, 8 and 9) responded well to treatment in that they did not show further

TABLE 1. Expression of HLA-DR antigen by keratinocytes in patients treated with interferon

Patient no.	Disease	Type of interferon	Response to treatment	HLA-DR + keratinocytes†
1	Melanoma	IFN-γ	Progression*	± (5 weeks)‡
2	Melanoma	IFN-γ	No progression**	+ (4 weeks)
3	Melanoma	IFN-γ	Progression	± (2 weeks)
4	Melanoma	IFN-γ	Progression	+ (3 weeks)
5	Renal carcinoma	IFN-γ	No progression	+ (5 months)
6	Melanoma	IFN-α_2	No progression	− (7 weeks) − (11 weeks)
7	Renal carcinoma	IFN-α_2	No progression	− (6 weeks) − (14 weeks)
8	Renal carcinoma	IFN-α_2	No progression	− (4 weeks)
9	Renal carcinoma	IFN-α_2	No progression	− (4 weeks) − (10 weeks)

* Clinical evidence of disease progression[32]

** No clinical evidence of disease progression[32]

† HLA-DR + keratinocytes: ± foci with faint HLA-DR expression; + foci with distinct HLA-DR expression

‡ Time between initiation of interferon therapy and biopsy.

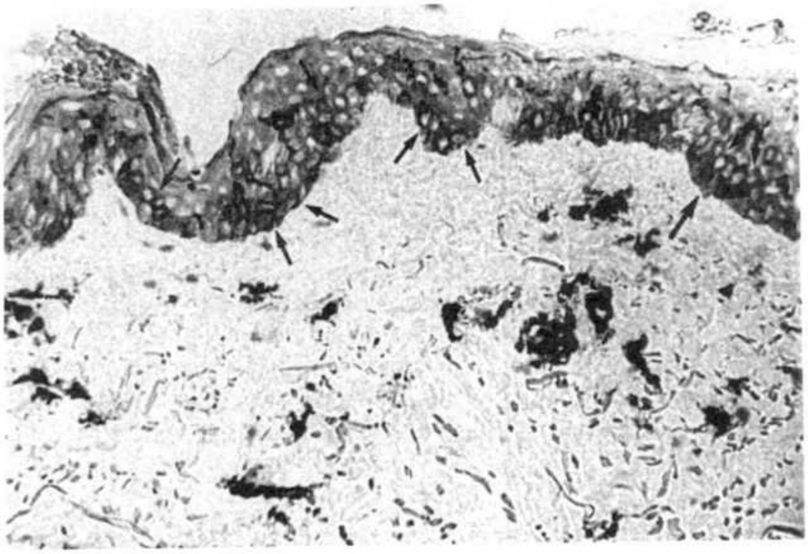

FIGURE 1. HLA-DR antigen expression by foci of basal and suprabasal keratinocytes (arrows); and by suprabasal dendritic Langerhans cells in a patient with metastatic melanoma treated with IFN-γ for 4 weeks. (Original × 150.)

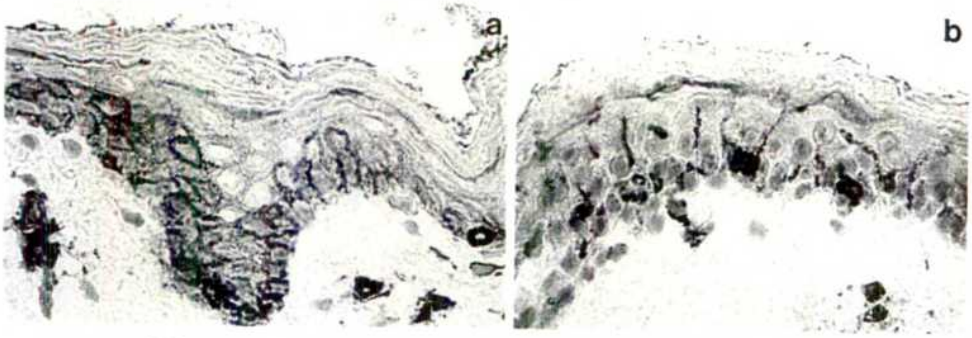

FIGURE 2. Higher magnification of Figure 1. (a) HLA-DR expression by basal and suprabasal keratinocytes. (Original × 600.) (b) Expression of HLA-DQ in the epidermis by dendritic Langerhans cells only. (Original × 600.)

tumour progression. Side-effects of the treatment consisted of fever, chills and headache (treated with oral paracetamol) and leukocytopenia.

After 4 weeks of treatment with IFN-γ, foci of HLA-DR expression were observed in the basal and suprabasal keratinocytes in four patients (Patients 1–4). This was more pronounced after 12 weeks and was then also seen in Patient 6 (Figs 1, 2a and 2b). Expression of HLA-DQ and HLA-DP was not observed.

In one patient (Patient 5) HLA-DR expression by keratinocytes was evaluated also 2 months after the last dose of IFN-γ. Class II antigens were not detectable on keratinocytes, while they were observed on epidermal Langerhans cells. VIC-Y1 reactivity, indicating γ-chain expression by keratinocytes, was not observed, either 4 or 12 weeks after initiation of IFN-γ treatment. This suggested that although HLA-DR antigens were induced and expressed on keratinocytes, the intracytoplasmic γ-chain was not induced. Class II MHC determinants were not observed on keratinocytes in patients treated with IFN-α_2.

DISCUSSION

This study indicates that systemically administered IFN-γ (in doses recommended for the treatment of advanced tumours) causes changes in cellular expression of class II alloantigens and leads to HLA-DR α and β chain expression by non-lymphoid cells, like keratinocytes.

The studies on the *in vivo* induction of class II antigens by IFN-γ on murine monocyte/macrophages as well as non-lymphoid cells like keratinocytes[13–15] contained no information on the induction of class II antigen subclasses. Our results point to HLA-DR α and β chain expression by keratinocytes, without simultaneous expression of HLA-DQ or HLA-DP and, interestingly, without apparent intracytoplasmic γ-chains. There has been much speculation about the possibility that the constant chain is necessary for the transport of class II α and β chains to the cell surface. Recent transfection experiments have shown that class II α and β chain transfection and subsequent expression on the host cells is not dependent upon the presence of the intracytoplasmic γ-chain (either the mRNA or the protein molecule).[21,22] We did not extract mRNA nor did we test for the presence of mRNA of the different class II moieties, but we believe that the three-step immunoperoxidase technique is sufficiently sensitive to demonstrate expression or lack of expression of class II molecules. Higher concentrations of the monoclonal antibodies also failed to stain these antigens. Antibody affinity does not seem to be responsible for our negative results because in certain pathological conditions the different class II moieties are found to be expressed.[23–25]

In view of these findings, it remains to be proven whether induction of HLA-DR expression by non-lymphoid cells like keratinocytes is a result of direct interaction between the sytemically administered IFN-γ and the target cells, or whether IFN-γ acts indirectly via activated T cells or monocytes which, upon activation, in turn release IFN-γ either alone or in conjunction with other lymphokines such as interleukin-4 (BSF-I).[26]

Furthermore, it is interesting to note that only basal and suprabasal keratinocytes express HLA-DR antigens; this indicates that only keratinocytes able to proliferate can respond to IFN-γ or engage in IFN-γ induced synthesis of HLA-DR antigens.

These findings add one more point to the complex issue of differential regulation of class II antigen expression, especially HLA-DR, -DQ and -DP antigens on the one hand and the γ-chain on the other. It is unclear whether this is simply due to the location of the γ-chain gene on chromosome 5[27] while the DR, DQ and DP genes are located on chromosome 6. The differential expression of HLA-DR α and β chains without co-expression of the intracytoplasmic γ-chain in keratinocytes after systemic administration of IFN-γ may be due to transient and tissue-specific control mechanisms operating at the molecular level. A class II regulatory gene outside the major histocompatibility complex has been postulated on the basis of studies of differential class II gene expression in patients with congenital severe combined immunodeficiency.[28] Similarly systemic IFN-γ may act via a regulatory gene or genes or may enhance elements which differentially regulate class II antigen expression.

The patients receiving IFN-γ and expressing keratinocyte-bound HLA-DR antigens showed neither clinical nor histological signs of adverse reactions. This indicates that HLA-DR α and β expression by non-lymphoid cells alone is apparently not sufficient to elicit an immunopathological response as long as T cells recognizing the additionally expressed class II antigens, alone or in context with hapten or antigen, are not present. However, this may have been, in part, due to the advanced stage of the tumours in our patients and the relatively short observation period.

The function of the aberrant HLA-DR antigens on keratinocytes remains open to speculation. They may serve as additional restriction elements required for the recognition by T helper lymphocytes[29] thus potentiating the initiation of the immune response. Class II antigens expressed by non-lymphoid cells may also contribute to induce tolerance against auto-antigens; since this phenomenon is both restricted to class II[30] and dose-dependent[31] sufficient aberrant HLA-DR antigens (or HLA-DR antigens without the additional expression of HLA-DQ antigens) may induce T suppressor cells, limiting autoimmune reactivity. Alternatively, keratinocytes expressing HLA-DR α and β chains may lack antigen-presenting capacity since they lack the intracytoplasmic γ-chains, to which at least some role in antigen presentation may be attributed.

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A clinicopathological study of mucosal involvement in linear IgA disease

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SUMMARY

Mucosal involvement in linear IgA disease was assessed clinically and immunologically using direct and indirect immunofluorescence (IF) techniques. There was clinical evidence of oral mucosal involvement in all 10 patients examined and conjunctival disease in six. Direct IF findings correlated well with clinical oral disease, with all patients demonstrating linear IgA deposits in the basement membrane zone of oral mucosa. However, this was not true of conjunctiva where no linear IgA could be demonstrated. Conjunctiva did provide a good substrate for indirect immunofluorescence using patients' sera, and showed that five of the 10 patients had circulating anti-basement membrane zone IgA.

Linear IgA disease (LAD) is a rare blistering dermatosis of unknown aetiology. It is characterized by a cutaneous eruption consisting of bullae which are often arranged in an annular configuration. The demonstration of a linear band of IgA deposited in the basement membrane zone (BMZ) of non-lesional skin is required for the diagnosis.

Initially LAD was considered to be a subgroup of dermatitis herpetiformis with implied gluten sensitivity,[1] but most authorities now recognise LAD as a distinct entity.[2,3] Although the disease is thought to have an autoimmune origin, circulating anti-BMZ antibodies are infrequently detected using conventional substrates in an indirect immunofluorescence (IF) method.[4] When antibodies are detected, titres are generally low and do not correlate with disease activity. Despite this, such antibodies are thought to be of pathogenetic importance. We have previously shown that the tissue used as substrate in indirect IF may influence the detection rate of circulating antibodies in bullous pemphigoid (BP) and cicatricial pemphigoid (CP).[5] In addition, simple techniques which separate tissue through the BMZ may be adapted to split substrates prior to use and this also provides a more sensitive assay of circulating anti-

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BMZ antibodies in some subepidermal bullous diseases. Tissue may be separated by a short incubation in 1 M NaCl, which provides a reproducible split through the lamina lucida.[6]

Clinical mucosal involvement has previously been documented in LAD[7] and in chronic bullous dermatosis of childhood (CBDC).[8,9] We have performed a clinicopathological study of mucosal involvement in 10 patients with LAD. This comprised clinical assessment, demonstration of immune complexes in skin and mucosae and detection of circulating anti-BMZ antibodies using a variety of substrates in an IF technique.

METHODS

Patients

Full details of the patients included in the study are given in Table 1. Ten patients (nine female and one male) were studied, all of whom had active disease at the time of the study. The age range was 19–73 years and the duration of disease 18 months to 20 years. In three patients the onset of the disease had been in childhood and had initially been diagnosed as CBDC[9-11] and, subsequently, as childhood cicatricial pemphigoid. Treatment consisted of either dapsone or sulphamethoxypyridazine (Lederkyn). All patients were assessed for extent of cutaneous, oral mucosal and conjunctival involvement.

TABLE 1. Clinical details of patients studied

Patient no.	Age (years)	Sex	Duration of disease (years)	Treatment
1	73	F	3	Sulphamethoxypyridazine
2	73	M	6	Prednisolone, dapsone
3	46	F	2	Dapsone
4	36	F	2	Dapsone
5	22	F	17	Dapsone, prednisolone
6	17	F	15	Dapsone
7	32	F	3	Dapsone
8	36	F	32	Dapsone
9	36	F	3	None
10	49	F	4	Dapsone

Tissues

Biopsies were obtained from clinically normal skin and oral mucosa from all 10 patients. Conjunctival biopsy was performed in seven patients. This took the form of a 2 mm snip excision which was taken from clinically normal bulbar conjunctiva lying beneath the upper lid.

The biopsies were divided, half being submitted for routine histopathological examination. The remaining tissue was snap frozen and stored in liquid nitrogen at −70°C until processed for direct immunofluorescence.

Serum

Serum was collected from all 10 patients, and stored at −70°C.

Tissue substrates

For indirect immunofluorescence studies normal tissue was obtained as follows: skin from healthy volunteers with no evidence of skin diseases, oral mucosa from patients undergoing minor oral surgical procedures, and bulbar conjunctiva from a redundant area of a corneal graft specimen. All tissue was divided, with part being snap frozen and stored in liquid nitrogen at −70°C. The remainder was incubated in 1 M NaCl to provide separation of the tissue through the lamina lucida. A modification of Scaletta's method was employed.[12] Thin strips of tissue were incubated in 1 M NaCl at room temperature for 24–48 h until the epidermis could be gently stripped from the underlying dermis. This provided a satisfactory separation for skin and oral mucosa, but not for conjunctiva, which proved technically impossible to split in this fashion. Thus separated, the tissue was snap frozen and stored in liquid nitrogen at −70°C. A representative section was cut from each tissue sample and stained with haematoxylin and eosin to confirm the orientation of the block, and adjacent 6 μm thick cryostat sections were cut and used as substrate for indirect immunofluorescence.

Immunofluorescence

Direct immunofluorescence. Standard immunofluorescence techniques were used.[13] For direct immunofluorescence 6 μm cryostat sections of frozen tissue were cut and washed in PBS at pH 7·4 for 20 min prior to a 30-min incubation with FITC labelled rabbit anti-human IgA, IgG or IgM (Dako). In addition three of the conjunctival biopsies were examined by FITC labelled mouse anti-human anti-secretory piece antibody.

Indirect immunofluorescence. For indirect immunofluorescence 6 μm cryostat sections of tissue substrates were cut from the frozen blocks. These were air dried for 15 min and rinsed in PBS at pH 7·4 for 15 min. Sections were then incubated at 37°C for 30 min with patient's serum, washed with PBS for 15 min and then incubated at 37°C for 30 min with FITC labelled rabbit anti-human IgG, IgM or IgA. After a final PBS wash, sections were mounted in buffered glycerol and then examined by ultraviolet microscopy.

Control patients

Clinically normal conjunctiva was obtained from redundant tissue from patients undergoing corneal transplants in the course of routine ophthalmological surgery.

Control sera

Ten sera chosen at random from healthy blood donors replaced test sera in control experiments.

RESULTS

Clinical

All 10 patients had clinical evidence of cutaneous disease activity. Oral involvement was also evident in all 10 patients. Principally, this took the form of blister formation, ulceration (Fig. 1) and erosion, with one patient having widespread erosive gingivitis and erosive cheilitis (Fig. 2). Examination of the eyes revealed conjunctival disease in six patients. The most severe cases showed extensive conjunctival adhesions between lid and globe (symblepharon) with secondary corneal opacification in one patient (Fig. 3). Two patients had focal symblepharon with trichiasis, but normal corneal appearance. One patient had loss of inferior forniceal depth. Two patients showed mild conjuctival abnormalities with moderate chemosis of the bulbar conjunctiva in one, and fine retropunctal tarsal conjunctival scarring in the other.

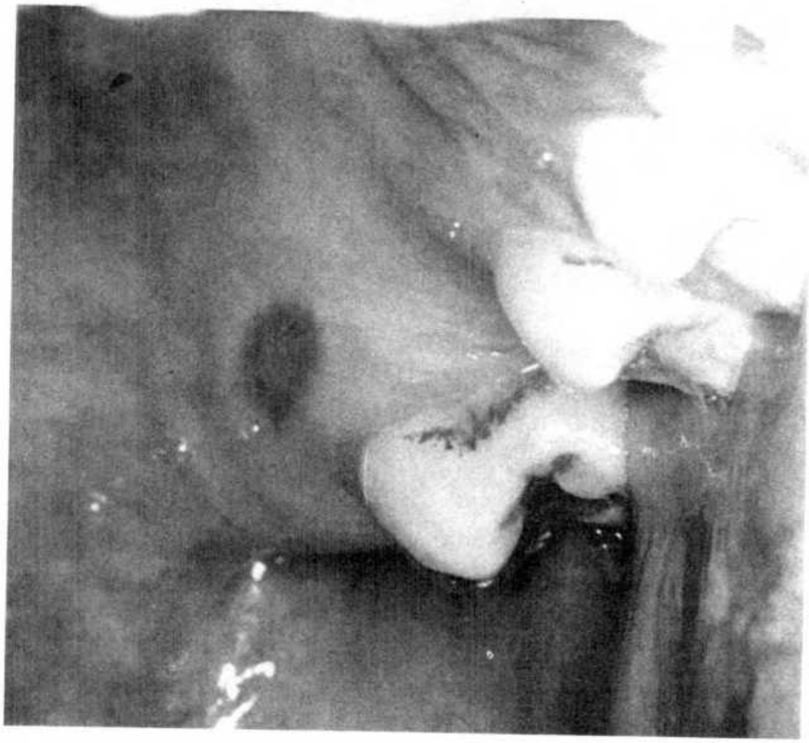

FIGURE 1. Ulceration of the buccal mucosa.

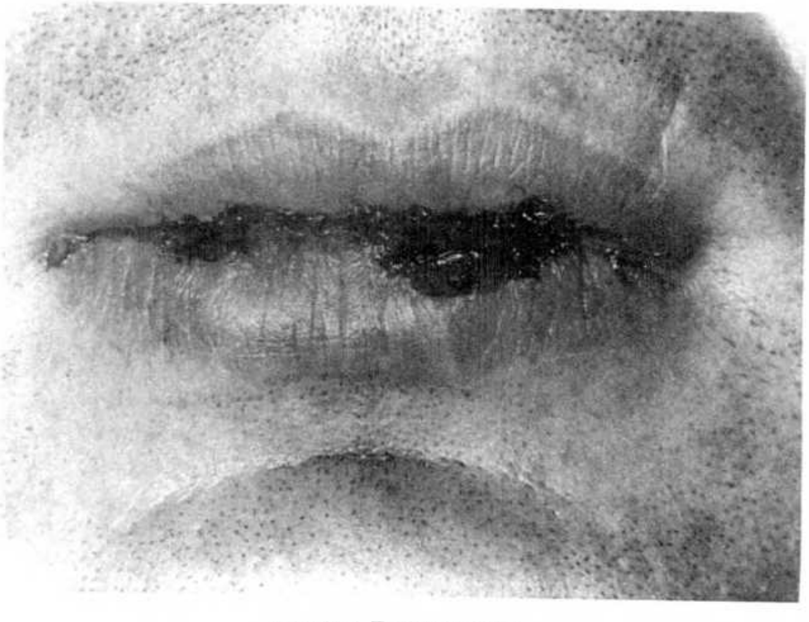

FIGURE 2. Erosive cheilitis.

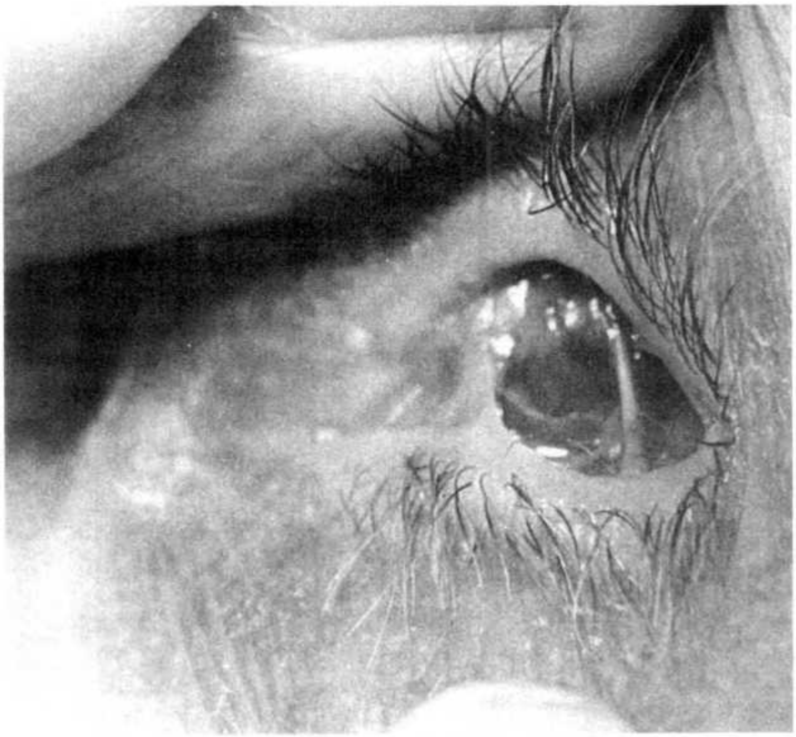

FIGURE 3. Symblepharon with secondary corneal opacification.

Direct Immunofluorescence

The results are summarized in Table 2.

Linear deposits of IgA were found in the skin of all the patients. Patchy deposits of IgG were detected in two of the skin biopsies.

TABLE 2. Results of direct immunofluorescence in 10 patients with LAD

Patient no.	Clinical involvement			Direct IF		
	Cutaneous	Oral mucosa	Conjunctiva	Skin	Oral mucosa	Conjunctiva
1	+	+	+	IgA, IgG	IgA, IgG	IgG
2	+	+	+	IgA	IgA	IgG
3	+	+	−	IgA, IgG	IgA, IgG	IgG
4	+	+	−	IgA	IgA	—
5	+	+	+	IgA	IgA	—
6	+	+	+	IgA	IgA	IgG
7	+	+	−	IgA	IgA	—
8	+	+	+	IgA	IgA	nd
9	+	+	+	IgA	IgA	nd
10	+	+	−	IgA	IgA	nd

All immunoglobulin was detected in a linear-BMZ pattern
nd = not done.

Linear deposits of IgA were detected in the oral mucosa of all 10 patients with deposits of IgG in two biopsies.

Conjunctival biopsies were performed in seven patients. Although 4 biopsies demonstrated linear deposits of IgG, no linear IgA deposits were seen in any of the biopsies. Deposits of IgA were, however, seen within the epithelial cells in five patients. No fluorescence was identified with the anti-secretory piece antibody preparation.

Indirect immunofluorescence

The results are summarized in Table 3.

Using normal human skin as substrate for indirect IF only two patients demonstrated a circulating anti-BMZ IgA. This immunoglobulin bound in a linear pattern along the length of the BMZ. When normal human oral mucosa was substituted as the substrate only one patient had circulating anti-BMZ antibodies (Fig. 4). However the use of normal human conjunctiva showed five patients, 50% of those studied, to have circulating anti-BMZ IgA (Fig. 5). Titres in general were low and ranged from 1:5 to 1:20.

TABLE 3. Results of indirect immunofluorescence in 10 patients with LAD

Patient no.	Substrate					Anti-BMZ antibody titre
	Skin	Oral mucosa	Conjunctiva	Split skin	Split oral mucosa	
1	IgA	–	IgA	IgA epidermal*	–	1:10
2	–	–	–	–	–	–
3	–	–	–	–	–	–
4	–	–	IgA	–	–	1:10
5	–	–	IgA	–	–	1:20
6	IgA	IgA	IgA	IgA epidermal*	IgA epidermal*	1:10
7	–	–	–	–	–	–
8	–	–	–	–	–	–
9	–	–	IgA	–	–	1:5
10	–	–	IgA	–	–	–

* Binding to epidermal side of NaCl split tissue

When normal tissue was replaced by chemically separated tissue, two sera demonstrated circulating IgA on split skin and one on split oral mucosa. These were the same sera which reacted with the intact tissue. All sera which bound to the separated substrate did so to the epidermal side of the split skin or oral mucosa (Fig. 6).

No circulating immunoreactant other than IgA was detected in any serum.

Controls

Conjunctival biopsies from patients undergoing routine eye surgery showed no specific linear staining with any immunoreactant. However, some of these patients did demonstrate patchy intra-epithelial IgA reactivity.

All control sera were negative on all substrates.

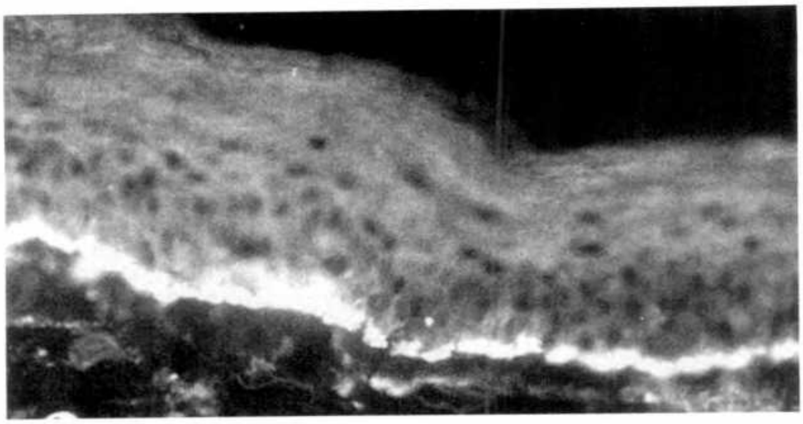

FIGURE 4. Indirect immunofluorescence showing linear IgA deposition in the BMZ of normal oral mucosa.

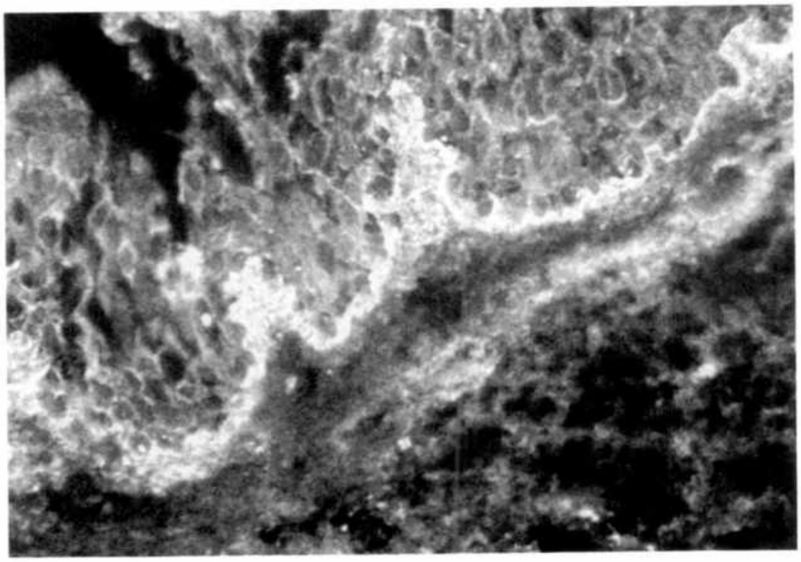

FIGURE 5. Indirect immunofluorescence showing linear anti-BMZ IgA, using normal conjunctiva as substrate.

DISCUSSION

We report here a clinical and immunopathological study of mucosal involvement in LAD. We found a high incidence of clinical mucosal lesions with all patients having oral disease and six out of 10 conjunctival disease. Leonard[7] detected evidence of oral ulceration in nine out of 34 patients with LAD and cicatrizing conjunctivitis in four out of seven.

Linear deposits of IgA were a consistent finding in all the biopsies of skin and oral mucosa, but were persistently absent in the conjunctival tissue. The IgA which was detected in the conjunctival biopsies was patchy and in an intraepithelial location. We regarded such deposits as non-specific, as an identical pattern of immunoreactant deposition was seen in normal controls.

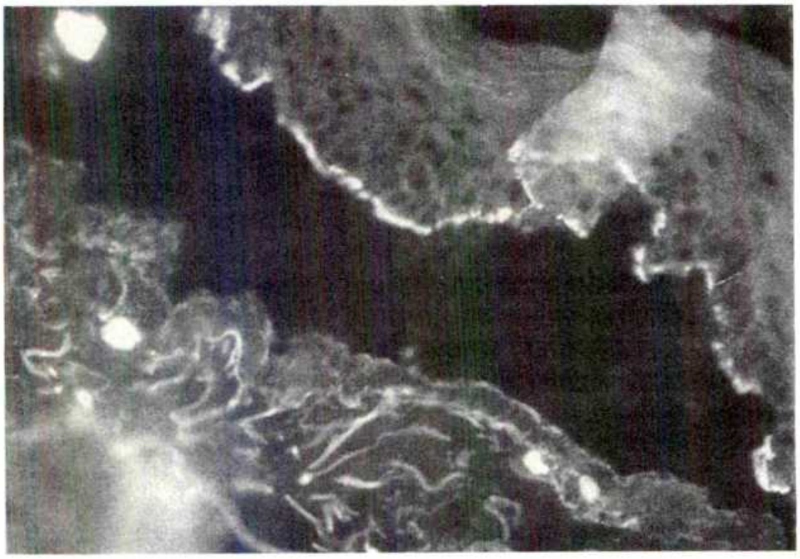

FIGURE 6. Binding of circulating anti-BMZ IgA to the epidermal side of 1M NaCl split skin.

We considered that the deposits of IgA which were observed in the superficial epithelium of conjunctiva had been adsorbed from tears.

Using conventional substrates for indirect IF we demonstrated a generally low incidence of circulating anti-BMZ antibodies. This confirms previous reports.[3,14] Unlike BP and CP where the use of chemically separated tissue as substrates increased the detection rate of circulating anti-BMZ antibodies in indirect IF, due to improved exposure of antigens which have been cryptic in intact substrates, no such increase in detection of anti-BMZ antibodies was seen in LAD.

Conjunctiva, however, did prove to be a highly sensitive substrate for indirect IF. Use of normal human conjunctiva in an indirect IF technique increased detection of circulating anti-BMZ antibodies in LAD to 50%. Such results also confirm that LAD antigen, as yet not fully categorized, is present within conjunctival epithelium.

However, clearly there is discordance between the demonstration of circulating anti-BMZ IgA and the lack of demonstrable IgA deposition in the conjunctiva of patients with LAD. IgA biochemistry and metabolism are complex and IgA molecules may exist in different forms. IgA1 and IgA2 subclasses are known, and monomeric and polymeric IgA forms have been detected.[15] There exists a distinctive distribution of subclasses and monomeric or polymeric forms in various body tissues and fluids. IgA does not activate the complement system by the classical pathway under biological conditions. In its polymeric form IgA contains the protein J chain, which is of plasma cell origin. It is the J chain which reacts with secretory piece, a membrane glycoprotein present in epithelial cells. The complex formed allows the transepithelial transport of IgA and its secretion.

We considered that any IgA present in the conjunctival biopsies may be present in a polymerized or secretory form and, therefore, might not be detectable by routine IgA IF. However, those conjunctival biopsies which were examined by anti-secretory piece antibodies did not show any evidence of secretory IgA in the BMZ.

Another possible explanation for the discordance between the results of direct and indirect IF is that the conjunctiva has processed the immunoreactant in some way, e.g. secreted the antibody-antigen complex into tears. Certain compartments of the eye are linked to the secretory immune system. IgA predominates in tears with a corresponding IgA-plasma cell predominance in the lacrimal gland.[16] Tissue in the ocular area appears to be directly linked to the common mucosal immune system, but it is also thought that a portion of serum derived antibodies appear in tears. Therefore, although most of the IgA immunoglobulin in the tears is thought to arise from active secretion from the lacrimal gland, some may be the result of trans-epithelial transport across the conjunctiva.

Linear deposits of IgG were detected, however, in four of the seven patients who underwent conjunctival biopsies. In bullous pemphigoid, immunoreactants are frequently detected in the conjunctival epithelium. Linear deposits of IgG and C_3 may be detected in up to 70% of patients with BP.[17] Furthermore, such immunoreactants seem to persist even when the disease is in remission. IgG may, therefore, be a more persistent immunoglobulin in conjunctiva, which may be detected even after IgA has been processed and cleared from the conjunctival epithelium. The finding that 50% of patients had circulating anti-BMZ antibodies of IgA class suggests that these antibodies are indeed pathogenetic.

ACKNOWLEDGMENTS

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Modulation by palmitoyl-carnitine of calcium activated, phospholipid-dependent protein kinase activity and inhibition of melanoma cell growth

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SUMMARY

Phospholipid-sensitive, Ca^{++}-dependent protein kinase activity was investigated in the cytosol of melanoma cells. A protein kinase system was partially purified, and enzyme activity was found to be modulated by palmitoyl-carnitine.

In order to link the actions of palmitoyl-carnitine on phospholipid-sensitive protein kinase activity and the already reported role of protein kinase C in cell division, we studied the action of palmitoyl-carnitine on melanoma cell growth by measuring colony forming ability in a soft agar culture system. Palmitoyl-carnitine was found to inhibit cell growth in a dose-dependent manner.

These findings suggest that palmitoyl-carnitine (or long-chain acylcarnitine), a naturally occurring metabolite, may play a key role in the onset of cell division.

We suggest that the action of palmitoyl-carnitine on phospholipid-dependent protein kinase activity is in part related to the molecular events linking protein kinase C activity and the ionic events in the initiation of cell growth.

Polypeptide hormones transmit information to the interior of the cell by activating transmembrane signalling systems that control the production of a relatively small number of chemical mediators.[1] These mediators behave as second messengers, translating external signals into internal signals. Recently, a crucial role in signal transduction for substances involved in cellular division was attributed to protein kinase C.[2] The existence of this phospholipid-sensitive Ca^{++} dependent protein kinase, which was first reported by Takai *et al*[2] is particularly significant.

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This enzyme has not only provided possible mechanisms for the action of Ca^{++}[3] and phospholipid, but also further expands conceptually the possibility of membrane generated 'second messengers' (diglycerides and phosphoinositides).[4] The possible relevance of protein kinase C activities to cell transformation and cancer has been suggested by the demonstration of cyclic nucleotide independent protein kinase activity associated with the product of viral genes.[5]

Agents which inhibit protein kinase C have already been investigated in various types of leukaemia and myocardial cells.[6,7] Among these inhibitory substances, L-palmitoyl-carnitine, a naturally occurring metabolite, is of major interest.[8] Several observations suggest that the accumulation of long-chain acyl-carnitines, i.e. amphiphile molecules, increases in ischaemic tissue and induces electrophysiological disturbances.[9,10]

On the other hand, it has been emphasized that ion-exchange is closely connected with the onset of DNA synthesis, and many workers are now investigating the ionic events controlled by protein kinase C.[11–14]

In the present study, we have examined the action of palmitoyl-carnitine on a partially purified phospholipid Ca^{++} dependent kinase extracted from melanoma cells. We also studied the action of palmitoyl-carnitine on the growth of these melanoma cells.

METHODS

Chemicals

Cyclic adenosine 3′5′ monophosphate (cAMP), 12-o-tetradecanoyl-phorbol,13-acetate (TPA), L-acetylcarnitine, L-carnitine, L-palmitoyl-carnitine, diolein, phosphatidyl-serine, histone H_1 (type IIIS), epidermal growth factor (EGF), and human insulin, were purchased from Sigma Chemical Co.

[^{32}P]-ATP (5000 Ci/mmol) was obtained from the Radiochemical Centre (Amersham, Bucks, U.K.) and DEAE-cellulose (DE 52) from Whatman.

Preparation of melanoma cell cytosol

Cutaneous tumours freshly obtained from an adult male patient were homogenized in three volumes of ice-cold 20 mM Tris-HCl buffer pH 7·5 containing 0·25 M sucrose, 10 mM EGTA, 2mM EDTA, 50 mM 2-mercaptoethanol and 0·1% Triton X100, using a Kinematika blender. This preparation was further homogenized by 50 strokes of a Pother-Eviljhem homogenizer.

The homogenate was centrifuged at 10,000 *g* for 10 min and the pellet was rehomogenized in two-thirds of the volume of the homogenization buffer.

The homogenate was combined with the initial supernatant and centrifuged at 105,000 *g* for 1 h and the supernatant used for further study.

DEAE-cellulose chromatography

The cytosol obtained from 5 g of fresh tissue was applied onto a DE 52 column, 1·5 × 10 cm, previously equilibrated with buffer containing 20 mM Tris-HCl, 5mM EGTA, 2 mM EDTA and 50 mM mercaptoethanol. After washing the column with 100 ml of the same buffer, elution was carried out with a 600 ml linear NaCl gradient (0 to 0·3 M) in buffer containing: 20 mM Tris HCl, 1 mM EGTA, 1 mM EDTA and 50 mM mercaptoethanol. An aliquot of each fraction (6 ml) was assayed for protein kinase activity.

Protein kinase

Protein kinase activity was assayed by measuring the incorporation of ^{32}P into H_1 histone from [^{32}P]-ATP. All reactions were carried out in polypropylene tubes.

According to the method of Takai,[15] the reaction mixture contained 5 μmol Tris-HCl, pH 7·5, 1·25 μmol magnesium acetate, 50 μg of H_1 histone, 125 μmol $CaCl_2$ (50 μl), 10 μg of phosphatidyl serine and 0·2 μg of diolein in a total volume of 0·25 ml.

The lipids had previously been dissolved in $CHCl_3$ and methanol and added to the reaction tubes. After the chloroform was removed under vacuum, the residue was suspended in 20 mM Tris-HCl by sonication.

In some experiments the diolein was replaced by TPA which was dissolved in DMSO to give a final concentration of TPA in the reaction tube of 10 μg/ml.[16] All reagents were made up in double distilled water which had been passed through a Chelax 100 column to remove as much Ca^{++} as possible.[17] [^{32}P]-ATP at a final concentration of 2 nmol/l was added. The reaction was started by the addition of an aliquot of eluant. The aliquot should not be excessive, in order to prevent EGTA and EDTA from completely complexing Ca^{++} and Mg^{++}. After incubation for 15 min at 30°C, the reaction was stopped by the addition of 25% trichloroacetic acid, and acid precipitable material was collected on a Sartorius nitrocellulose membrane filter (pore size 0·45 μm).

Activity of protein kinase C as a function of palmitoyl-carnitine concentration

Phosphorylation was performed under the standard conditions described above, the palmitoyl-carnitine being first added to the reaction tubes in methanol solution and evaporated under vacuum.

The curve was linearized by plotting. $\mathrm{Log}_{lt}Z = \mathrm{Log}\ Z - \mathrm{Log}(1-Z)$ against Log palmitoyl-carnitine where $Z = Y/Y_{max}$, $Y = \mathrm{cpm}\,^{32}P$ incorporated and Y_{max} = maximum ^{32}P incorporated. This logistic model is equivalent to a Hill-plot.[19]

Measurements

The radioactivity of the ^{32}P samples was determined using a Packard-Tri-Card liquid scintillation spectrometer, model 3520.

Protein was measured by the method of Bradford[18] with bovine serum albumin as a standard.

Protein phosphatase assay

P-nitrophenyl phosphatase activity was measured by recording the change in absorbance at 410 nm. The assay mixture at 30°C contained 0·1 M Tris-HCl, pH 9·0, 5 mM paranitrophenyl phosphate, and an aliquot of enzyme (25 μl) in a total volume of 1·0 ml.[20]

Cell cultures.

Tumour cells. We used the melanoma cells described above, and a melanoma cell line CAL-1 isolated from a bone metastasis by Dr Gioanni (Centre A. Lacassagne, Nice, France). The cells were grown in Corning 25 cm^2 tissue culture flasks in 5 ml Dulbecco's modified Eagle's medium.

The cells were incubated in a humidified atmosphere containing 5% CO_2 at 37°C for 7 days. They were then removed from the culture flasks by enzymatic disaggregation (using trypsin 0·05% and EDTA 0·02%). Cell viability was determined by trypan blue exclusion.

Soft agar colony forming assay

The human tumour cell line was cultured in soft agar using a modification of the double layer agar system described by Hamburger and Salmon.[21]

The lower layer consisted of 1 ml 0·5% agar in enriched RPMI 1640 medium. The upper layer consisted of 1 ml 0·3% agar in RPMI medium containing 5 × 10^3 cells.

Colonies were counted using an inverted microscope 14 days after plating. All experiments were performed at least three times in triplicate.

Drugs were incorporated in the upper layer at the following final concentrations: L-carnitine chloride (Boehringer-Mannheim, FRG) 0·1 μg/ml to 10 mg/ml, palmitoyl-carnitine chloride (Sigma) 0·2 μg/ml to 200 μg/ml, epidermal growth factor (Sigma) 0·001 ng/ml to 100 ng/ml, vanadate 0·4 nM to 400 nM, and insulin (Actrapid) 0·0001 IU/ml to 4 IU/ml.

The drug effects were measured by counting the colonies produced and expressing this as a percentage of the number of colonies in control drug free cultures. Sensitivity to a drug was defined as less than 30% colony formation.

RESULTS

Characterization of a Ca^{++}, phospholipid-dependent protein kinase in the cytosolic fraction of melanoma cells

Figure 1 illustrates the elution profile of protein kinase activity measured when the melanoma cell cytosol (17·5 mg protein) was analysed by DEAE-cellulose chromatography.

The major activity appeared between tubes 12 and 20 (at about 0·05 M NaCl). This activity was not influenced by the presence of cAMP (1 μM). An additional peak of activity was also present between tubes 52 and 60.

Histone kinase activity was measured in the presence and absence of Ca^{++}, phosphatidyl serine and TPA or diolein. The first peak of activity was strictly phospholipid-dependent and no activity could be detected in the absence of Ca^{++} and phosphatidyl serine. The phosphorylation reaction was linear with respect to the amount of enzyme and the incubation time for all experiments. The kinase activity of the second peak remained unchanged when Ca^{++} and phosphatidyl serine or diolein were omitted from the reaction tubes. No p-nitrophenyl

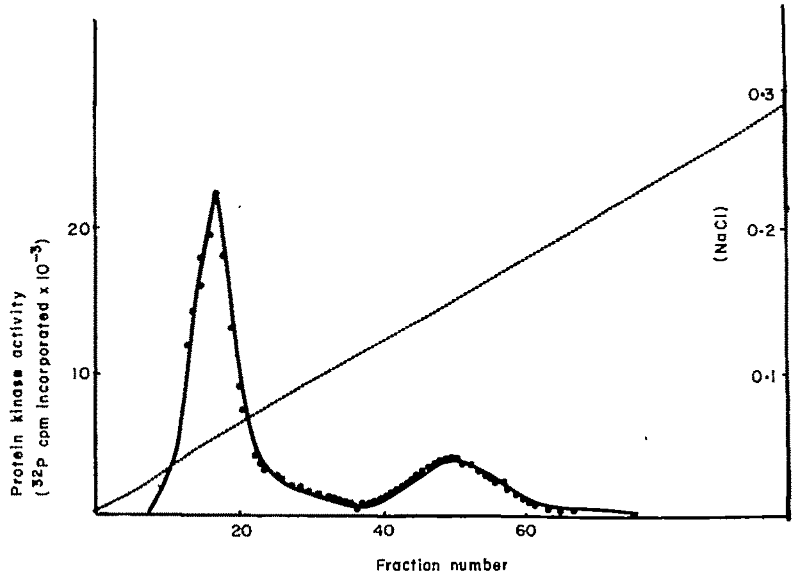

FIGURE 1. DEAE cellulose (DE 52) column chromatography of protein kinase from melanoma cells. Solid line indicates protein kinase activity and dashed line NaCl concentration.

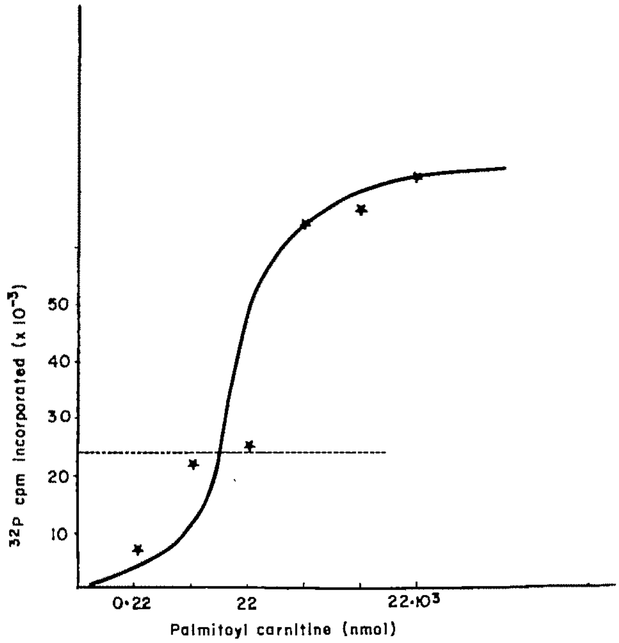

FIGURE 2. Activity of protein kinase in relation to the concentration of palmitoyl-carnitine. Each point represents the mean of two independent experiments. Half maximum phosphate incorporation was obtained with 55·7 nmol palmitoyl-carnitine. Dashed line indicates control ^{32}P incorporation in the absence of palmitoyl-carnitine.

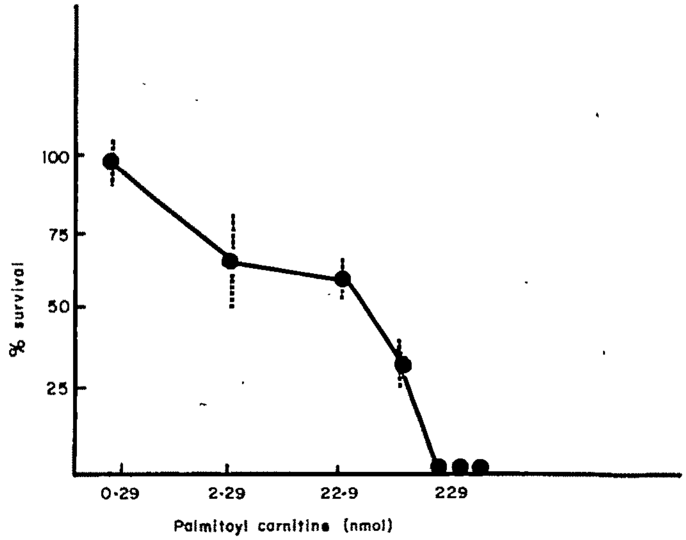

FIGURE 3. Inhibition of cell growth by L-palmitoyl-carnitine. Points are means ± SEM of seven sets of triplicate experiments. A spline-fit curve[33] enabled the determination of the LD$_{50}$ as 63·45 nmol/ml.

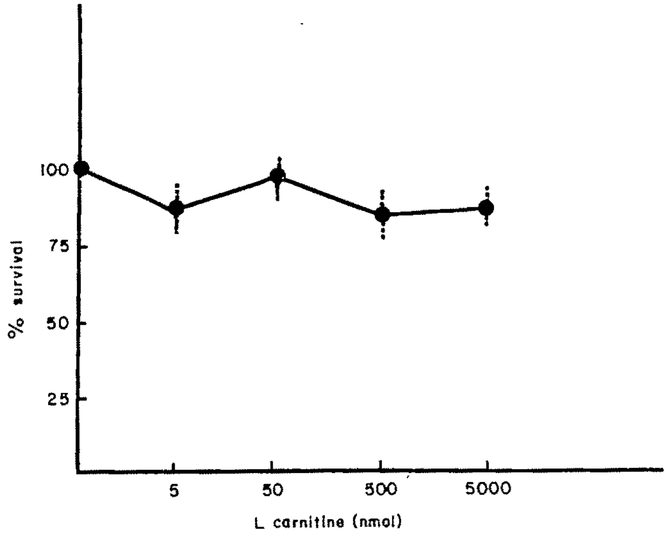

FIGURE 4. Colony forming assays in the presence of L-carnitine. Points are means ± SEM of five different experiments.

phosphatase activity could be detected between tubes 12 and 20. This elution profile of enzyme activity was comparable to those reported by others in different tissues.

It was observed that in crude homogenates, in the same experimental conditions, no histone H_1 kinase activity could be detected. Therefore, an appreciable increase in activity could be accounted for by partial purification, fractionation and ion-exchange chromatography.

We next examined the possible regulation by L-palmitoyl-carnitine of the phosphorylation activity of the first peak. We found that palmitoyl-carnitine acted in a dose-dependent manner on the phosphorylation activity (Fig. 2).

L-palmitoyl-carnitine caused a partial inhibition of phosphorylation activity when the concentration was below 22 nmol/ml, but the activity was strongly enhanced above this concentration, then reaching a plateau; thus the action of palmitoyl-carnitine followed a sigmoïdal curve. Neither L-acetylcarnitine nor L-carnitine had an effect on phosphorylation.

Soft agar colony forming assays

Before examining the effects of L-palmitoyl-carnitine, we investigated the possible sensitivity of melanoma cells to growth factors.

The addition of exogenous growth factors when the culture medium contained growth factors was investigated in the hope that this could stimulate the growth of the melanoma cells. 'Progression' growth factors (i.e. insulin and EGF) had no stimulatory effect (results not shown).

After subjecting the melanoma cells to various concentrations of palmitoyl-carnitine, the capacity of the cells to give rise to new colonies was assayed.

There is a close relationship between cell growth inhibition and the amount of palmitoyl-carnitine added to the medium (Fig. 3). L-carnitine on the other hand was not inhibitory (Fig. 4).

In order to test whether L-carnitine was antagonized by palmitoyl-carnitine (dietary requirement of carnitine: 50 μmol/l), the medium was supplemented with L-carnitine in a third group of assays. L-carnitine could not restore the growth of the cells in the presence of L-palmitoyl-carnitine.

DISCUSSION

Melanoma cells are strictly serum-dependent and this need for serum probably reflects their dependence on serum growth factors.[22-25]

No mutation leading to growth factor independence has been reported in melanoma cells and we did not succeed in cultivating them in a serum-free medium.

L-palmitoyl-carnitine was found to be a potent inhibitor of cell growth as assessed by the colony forming assays. It should be emphasized that the concentration actually reported for the plasma long-chain and short-chain acylcarnitines is 22 nmol/ml.[26]

In our experiments the LD_{50} of L-palmitoyl-carnitine was 63·45 nmol/ml. Cell growth inhibition could also be obtained with smaller amounts.

The tissue distribution of long-chain acylcarnitines has been studied thoroughly by many workers. The concentrations of these long-chain derivatives of carnitine are higher than those required for melanoma cell inhibition.[26,27] Among the different targets of this metabolite, a phospholipid-dependent protein kinase seems to be one of the most interesting, as assessed by histone H_1 phosphorylation assays.

The elucidation of the dual effects of palmitoyl-carnitine raises the question of the possible existence of a phosphoprotein phosphatase which could dephosphorylate histone H_1[28] and be, in turn, another target of palmitoyl-carnitine. This is of course speculative, but at the present time, neither the existence of a protein kinase C inhibitor, nor a potent phosphoprotein phosphatase that would antagonize the phosphorylation of histone H_1 has been identified in melanoma cells.

Recently, Yuan and Sen[29] emphasized the possibility of protein kinase undergoing autophosphorylation. They isolated from canine cytosol, an endogenous phosphorylated protein of mol. wt. 78000, which could be protein kinase C.[29] Wise and Kuo[8] reported that L-palmitoyl-carnitine inhibited a phospholipid Ca^{++} sensitive protein kinase by competing with phosphatidyl serine. They suggested that a stoichiometric interaction might exist between the two lipids. They insisted that the inhibition did not obey classical Michaelis-Menten kinetics.

The reported actions of L-palmitoyl-carnitine on Na^+-K^+-ATPase should also be considered[30-32] and cell-growth inhibition may be partly attributable to this inhibition.

The functional role of protein kinase C remains unclear. The available evidence suggests that the pleiotropic actions of tumour promoters are mediated through this protein kinase. It is tempting to suggest that modulation of protein kinase activity by palmitoyl-carnitine may be one of the ways in which this substance acts on cell growth.

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Preliminary identification of a low molecular weight serological mediator in chronic idiopathic urticaria

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SUMMARY

Sixteen patients with chronic idiopathic urticaria were skin tested with their own serum, 10 with autologous plasma and five with serum that had been heated to 56°C to inactivate complement. Eight showed a weal and flare response to whole serum, four to plasma and five to heat-treated serum. All serum-positive patients showed the same response to their own plasma and to heated serum, indicating that the mediator concerned is not generated by clotting and is not dependent on a functioning complement pathway. Three control subjects were negative to autologous serum, plasma and heated serum. Local tachyphylaxis was demonstrated in five serum-positive patients on reinjection of the same site with autologous serum on 3 consecutive days. This raises the possibility that the serological mediator may be acting by mast cell degranulation or directly on receptors in blood vessels and that repeated injections could induce a change in the number of receptors. Passage of whole autologous serum from four serum-positive patients through ultrafiltration membranes showed that fractions with a molecular weight of less than 30,000 daltons were still able to produce a positive skin test response, but those less than 1000 daltons were not. All serum fractions from two serum-negative patients and three normal controls were negative. Whole autologous serum from five serum-positive patients and two control subjects was separated by column chromatography. On skin testing with pooled fractions, the greatest response was produced by fractions of 10,000–15,000 daltons in the serum-positive patients, but there was no response in the controls.

A previous study[1] provided evidence of a serological mediator in some patients with chronic urticaria. It was found that autologous serum produced a weal and flare on intradermal reinjection, which was evident within minutes and was maximal at between 1 and 2 h. Histology of skin tests showed a predominantly neutrophilic infiltrate within and around dermal

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capillaries. The cellular response to normal saline, on the other hand, was mainly mild and mononuclear. It was also shown that the serological mediator was present while the urticaria was active, but disappeared after remission. The purpose of the present study was to determine the molecular weight of the mediator to exclude the possibility that it was in fact an *in vitro* clotting artefact.

METHODS

Patients

Sixteen patients, (six males, 10 females, mean age 44 years, range 17–76 years) with chronic urticaria of more than 2 months duration took part in the study. The mean duration of urticaria was 2·2 years (range 2 months–20 years). Patients with predominantly physical urticarias, urticarial vasculitis, or hereditary angioedema were excluded by appropriate history taking, examinations, a blood screen including antinuclear antibody, hepatitis B surface antigen, full blood count and a biochemical profile and, occasionally, a skin biopsy.

Skin test technique

All skin tests were performed on volar forearm skin. Patients discontinued antihistamines at least 48 h beforehand. Injections (100 μl) were given intradermally, accompanied by an adjacent control injection of normal saline on each occasion. Up to four skin tests were placed on each forearm, separated by about 5 cm. The siting of the skin tests was not randomized and generally followed the same order. For the pooled column fractions this ranged from the highest to the lowest molecular weight. The mean diameters of weal and erythema for each skin test were recorded after 1 h and 2 h. A positive response was defined as a weal at least 5 mm greater than the saline control, and erythema at least 10 mm greater than the control within 2 h of injection. It should be noted that these criteria were chosen to separate strong from weak responses and the definitions of positive and negative responses used here are arbitrary values chosen for convenience.

The intensity of the skin test response to pooled column fractions was taken as the sum of the mean diameters of weal and erythema, measured in mm, taking the highest value over the 2 h observation period. The results were also corrected for the volume effect of injecting normal saline by subtracting the respective diameters of weal and erythema in an accompanying saline control skin test.

Skin tests

All patients were skin tested with their whole serum. Venous blood was allowed to clot in sterile glass bottles at room temperature and separated by centrifugation within 1 h. A small aliquot of fresh serum was used for the initial skin test and the rest was stored at −70°C.

Ten patients were skin tested with their fresh plasma. Whole blood was anticoagulated with sodium heparin (5 U/ml), and the plasma separated by centrifugation and used for skin testing without freezing.

Five patients were also skin tested with whole serum which had been incubated at 56°C for 30 min to inactivate complement.

Local tachyphylaxis

In five patients, skin tests with whole serum were repeated at the same site (Site A) on 2 consecutive days. An adjacent site (Site B) was injected with normal saline. On the 3rd day serum was injected at both the serum and saline treated sites.

Ultrafiltration

Whole serum from six patients (4 serum-positive and 2 serum-negative) and three normal control subjects, was filtered through Amicon® YM2 and YM30 membranes, which allow passage of molecules up to 1000 and 30,000 daltons, respectively. A 2 ml volume of stored serum was introduced into an Amicon® minicell and filtered at 4°C under positive pressure using compressed air at 3·5 atmospheres until approximately half the volume had passed through. A fresh membrane was used for each sample and the apparatus was autoclaved between patients to prevent the possibility of transmission of infection. Both filtrates and concentrates were collected, stored at −70°C and sterilized by filtration through 0·2 μm pore size disposable filters before skin testing.

Column chromatography

Whole sera from five serum-positive patients and two normal control subjects were separated by column chromatography. Sephadex G75 gel was swollen with sterile isotonic saline and packed into a 100 ml column. A new gel was freshly prepared for each patient and the column was autoclaved before packing. A 5 ml volume of serum was eluted down the column with normal saline at a flow rate of 1 ml/min. Three consecutive 5 ml fractions were pooled into groups (15 ml), stored at −20°C and sterilized by filtration immediately before use.

In one additional patient, the column separation was performed with 1 ml of whole serum and 1 ml fractions were collected; 0·1 ml aliquots of these were pooled in groups of 10 for screening skin tests. Fractions were then pooled in twos over the range showing the greatest skin test response, for further testing.

RESULTS

Skin tests with whole serum, plasma and complement-inactivated serum

Eight of 16 patients showed a positive response to their whole serum according to our criteria. Only two showed no greater response to serum than to saline; one of these was going into

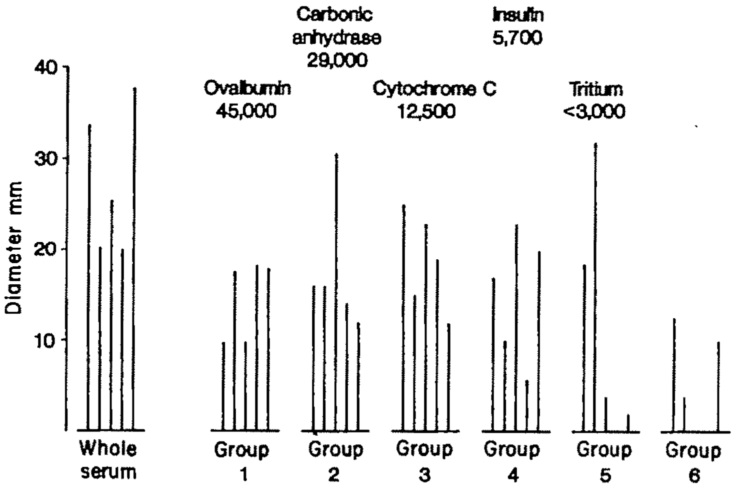

FIGURE 1. Skin test responses in individual patients to Sephadex G75 column fractions of serum. Values are maximum diameters of weal and erythema measured during the 2 h observation period, corrected for the saline control. Molecular weight standards corresponding to each group of fractions are also indicated.

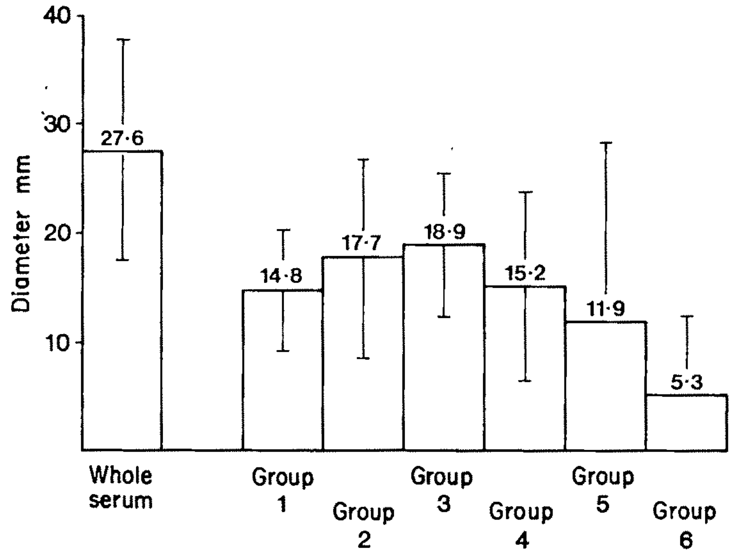

FIGURE 2. Skin test responses to Sephadex G75 column fractions of serum. Values are means ± 95% confidence intervals of results in five patients for maximum diameters of weal and erythema measured during the 2 h observation period, corrected for the saline control.

remission at the time of the skin test and the other had had continuous chronic urticaria for 20 years. Four of 10 patients reacted to their plasma and all the five patients tested reacted to heat-treated serum. All serum-positive patients reacted to autologous plasma and to heat-treated serum, but no serum-negative patients reacted to autologous plasma. One patient who was negative to whole autologous serum showed a weakly positive response to his heat-treated serum. There were no reactions to autologous serum, plasma or heat-treated serum in the three control subjects.

Local tachyphylaxis

Local tachyphylaxis was demonstrated in five patients. Site A reacted to serum on day 1 but not on days 2 and 3. By contrast, the control site B showed no response to saline on days 1 and 2, but produced a strong reaction to serum on day 3.

Ultrafiltration

None of the six patients reacted to the YM2 filtrates, indicating that the biologically active mediator had a molecular weight greater than 1000 daltons. However, three of the four patients who were positive to whole serum reacted to the YM30 filtrate, indicating that the mediator, or at least one component of it was capable of crossing the membrane and was, therefore, less than 30,000 daltons in molecular weight. Concentrates from both membranes produced reactions in all serum-positive patients and some serum-negative patients.

Column chromatography

The magnitude of the individual skin test responses for each patient with each group of pooled fractions is shown in Figure 1. It can be seen that the responses dropped off sharply below 3000 daltons. The means for each group are shown in Figure 2. It can be seen that the greatest response occurred with Group 3, corresponding to the cytochrome c marker.

When the column was loaded with only 1 ml of whole serum from another serum-positive patient and 1 ml fractions were collected, there was a peak of response on skin testing between 24,000 and 30,000 daltons.

DISCUSSION

Our results show that an inflammatory mediator can be detected in the serum and plasma of some patients with chronic urticaria by its biological activity on skin testing. Activity was not lost on freezing or by heating to 56°C, indicating that the skin test response is not dependent on a functioning complement pathway. The ability to induce local tachyphylaxis suggests that the mediator may be acting by mast cell degranulation or, alternatively, that it may act on receptors in blood vessels and that repeated injections induce a change in the number of receptors.

Fractionation by ultrafiltration and column chromatography showed that the mediator had a molecular weight in excess of 1000 daltons, thus excluding histamine and products of arachidonic acid metabolism. Data on the pooled fractions are difficult to interpret because the 95% confidence intervals for the groups are wide, but the results suggest a peak of activity within the molecular weight range 10,000–15,000 daltons. The finding of a peak at 24,000–30,000 daltons in the last patient could be due to there being more than one mediator. Alternatively the mediator might exist in a dimeric form. The fairly wide range of responses in individual patients shown in Figure 1 may have been due to overloading of the column because the peak was much sharper in the last patient when only 1 ml of serum was used.

C_{5a} anaphylatoxin has a molecular weight of 11,000 daltons and has been shown by other workers to produce a weal and flare reaction with a similar time course to that found in the present study, on skin testing.[2] It also causes mast cell degranulation and neutrophil accumulation *in vivo*. Further studies are, therefore, required to look for C_{5a} activity in grouped column fractions and whole sera.

ACKNOWLEDGMENTS

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Ocular findings and skin histology in a group of patients with X-linked ichthyosis

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SUMMARY

We carried out ophthalmological examinations, and histopathological examinations of skin biopsies in 32 male patients affected by X-linked ichthyosis.

We found corneal opacities in only five patients, and their presence was not related to the age of the patients. Skin histology revealed a reduction of the granular layer in nine cases, a finding previously thought to be typical of autosomal dominant ichthyosis vulgaris.

Wells and Kerr[1] first proposed a genetic classification of ichthyosis. They showed that X-linked ichthyosis (XLI) could be differentiated from autosomal dominant ichthyosis vulgaris by the typical features of the scales (large and dark, localized to the neck, trunk and extremities, sparing the palms and soles and improving during the summer), by the frequent association with corneal opacities, and by skin histology (normal or increased granular layer and hyperkeratosis). In 1978 the biochemical basis of XLI was shown to be a deficiency of steroid sulphatase (STS).[2] STS is a microsomal enzyme that plays an important role in the metabolism of oestrogen and cholesterol sulphate.[3]

Besides being associated with skin abnormalities, STS deficiency is related to a history of delayed delivery.[3] More recently, a significant number of patients were reported with gonadal dysfunction, including cryptorchidism, infertility, micropenis and small testes,[4] and some further cases also showed features of Kallmann syndrome (hypogonadotropic hypogonadism and anosmia).[5–7]

Two research groups recently cloned an STS cDNA and showed the frequent occurrence of gene deletions in STS deficiency.[8,9] Thus, the associated clinical features observed in some patients with XLI might be explained by deletions involving other genes adjacent to the STS locus. This seems to be the case at least in the families in which XLI and Kallmann syndrome occurred together.[10] In the present study we have reassessed the frequency of ocular abnormalities and the features of the skin pathology in 32 patients from 27 families, with

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TABLE 1. Ocular findings and skin histology in patients with X-linked ichthyosis

Patient no.	Age (years)	Corneal opacities†	Stratum granulosum	Diagnostic features‡
1	2	−	normal	cDNA
2	3	−	normal	STS
3*	4	−	normal	STS, lipo
4	4	−	normal	cDNA
5**	6	+	reduced	STS
6	7	−	normal	STS, lipo
7**	7	−	reduced	STS
8	8	−	increased	STS
9	9	−	reduced	cDNA
10	10	+	normal	STS
11	11	−	increased	STS
12	12	−	normal	cDNA
13	15	+	normal	cDNA, lipo
14	15	−	reduced	STS
15	17	−	reduced	cDNA, lipo
16	19	−	normal	STS
17	20	−	reduced	lipo
18	20	−	normal	lipo
19	22	−	reduced	STS
20	22	−	normal	cDNA, lipo
21	24	−	reduced	STS, lipo
22***	24	−	normal	lipo
23	25	+	reduced	STS
24*	25	+	normal	cDNA, lipo
25	26	−	normal	STS
26	28	−	normal	cDNA
27*	28	−	normal	cDNA, lipo
28***	30	−	increased	cDNA
29	31	−	normal	cDNA
30	32	−	normal	cDNA
31	33	−	normal	lipo
32	38	−	normal	cDNA, lipo

Patients belonging to the same family *Family 1; **Family 2; ***Family 3

†Corneal opacities: + present; − absent.

‡STS = STS deficiency in cultured fibroblasts; cDNA = lack of hybridization of patient's DNA to a specific STS cDNA probe

lipo = altered electrophoretic mobility of plasma lipoproteins.

documented STS deficiency and compared these results with those obtained in 10 patients from eight families with autosomal dominant ichthyosis.

METHODS

Patients

We studied 32 patients with XLI from 27 families; their ages ranged from 2 to 38 years. The diagnosis was confirmed by demonstrating steroid sulphatase deficiency in cultured skin fibroblasts, according to the method described by Piraud *et al.*,[11] lack of hybridization of patient's DNA with a specific STS cDNA probe in the Southern blotting technique[8] and electrophoretic mobility of plasma lipoproteins (according to the method described by Epstein *et al.*[12]) In many cases two of these diagnostic features were investigated (Table 1).

Clinical, biochemical and molecular studies on some of these patients have been reported elsewhere.[5,6,10]

Ten patients with autosomal dominant ichthyosis vulgaris from eight families were also studied for comparison. Assay of STS activity in fibroblasts from these patients gave normal results.

Ophthalmological examination

Ophthalmological examinations were carried out by two expert ophthalmologists using a Haag-Streit slit lamp.

Histology

A skin biopsy was obtained using a 4 mm punch from the posterior aspect of the upper arm, an area commonly and often markedly involved by ichthyosis. The specimens were stained with haematoxylin and eosin.

RESULTS

The results obtained in the patients with XLI are summarized in Table 1. Five patients, with ages ranging from 6 to 25 years had corneal opacities. The opacities were found in one layer of the cornea, at the level of the Descemet membrane, or in very deep stroma, and were uniformly distributed as small opacities over the whole cornea. No other corneal or conjuctival alterations were present. Corneal opacities were not detected in any patients with autosomal dominant ichthyosis. Reduction or absence of the granular layer was observed in the skin biopsies of nine patients with XLI and in all the patients with autosomal dominant ichthyosis.

DISCUSSION

X-linked ichthyosis is described in the literature as being characterized by hyperkeratosis, a normal or slightly thickened granular layer[13,14] and the common occurrence of corneal opacities.[15]

We carefully studied 32 patients affected by XLI and found only five patients with ocular abnormalities, consisting of small dots of corneal opacity. Corneal opacities were absent in most patients over 20 years of age.

Previous studies suggested that corneal opacities are not consistently associated with XLI, as first reported by Sever *et al.*[15] and Barrie *et al.*,[16] who found ocular changes in 13 out of 26 patients with XLI. These authors proposed that the age of the patients might be of importance, the ocular abnormalities being uncommon under the age of 10 and invariably present over the age of 25.

More recently, Lykkesfeldt *et al.*,[17] reported ocular changes in 14 out of 28 patients affected by XLI. In this study no relationship between the age of patients and the occurrence of corneal opacities was found. The pathological findings in our patients also seem to call into question another feature considered to be useful in the diagnosis of genetic ichthyosis. Reduction or absence of the stratum granulosum, found in all patients with autosomal dominant ichthyosis, was also observed in nine of our 32 patients with XLI.

We believe that a differential diagnosis between XLI and autosomal dominant ichthyosis, especially in sporadic non-familial cases, should rest essentially on the demonstration of STS deficiency. Clinical features can of course be of help, but ocular and histopathological findings, at least, do not seem to be absolute criteria for a definite diagnosis.

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Staphylococcal colonization in atopic dermatitis and the effect of topical mupirocin therapy

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SUMMARY

Forty-nine patients with atopic dermatitis entered a double blind placebo controlled cross-over study of mupirocin, a new topical antistaphylococcal antibiotic. Forty-five patients were evaluable. Quantitative bacteriological assessment before treatment showed that heavy colonization of the skin with *Staphylococcus aureus* was present in nearly all patients even in the absence of overt infection. However, the bacterial count was significantly reduced by 2 weeks' treatment with topical mupirocin, but not by the placebo. Moreover, a significant reduction of clinical severity was also observed after treatment with mupirocin, which was maintained over the following 4 weeks, although recolonization occurred during this period, with bacterial counts rising to pre-treatment levels. Despite recolonization, clinical deterioration was not observed during the trial period. No serious side-effects were observed.

Phage typing showed that 50% of patients carried more than one bacterial phage type. Recolonization in eight patients (17%) was with a 'new' strain that had not previously been isolated.

Chronic atopic dermatitis may be aggravated by a number of factors. Cutaneous infection, usually with *Staphylococcus aureus*, is one such factor and systemic antibiotics play a major role in the treatment of exacerbations. Heavy skin colonization with *S. aureus* has been reported in patients with atopic dermatitis even if the skin is not clinically infected,[1–4] and this may contribute to continuing disease activity.[5] Patients with atopic dermatitis may show increased adherence of staphylococci to both skin and mucous membrane surfaces [6–9] and *S. aureus* may have specific binding sites, possibly protein A or teichoic acid, on the cell wall, for fibronectin[10] and laminin[11] in the tissues.

Although antibacterial activity of *Pseudomonas fluorescens* was demonstrated almost a century ago, antimicrobial metabolites have only recently been isolated and developed commercially. Mupirocin (Bactroban) has a novel mode of action in that it inhibits the enzyme isoleucyl

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transfer-RNA synthetase and thus stops bacterial protein synthesis.[12] It is rapidly metabolized systemically and is excreted mainly as the biologically inactive monic acid in the urine. It has a wide range of activity against Gram positive bacteria and is active against staphylococci, including methicillin resistant *S. aureus*.[13]

The present study was undertaken to assess the degree of skin colonization with *S. aureus* in patients with atopic dermatitis and to determine how this changed with topical therapy with 2% mupirocin ointment. Concomitant changes in the clinical severity of the dermatitis were also recorded.

METHODS

Clinical details

Patients aged 2 years or older were included in the study provided they had the typical clinical features and classical relapsing course of atopic dermatitis. They were excluded if systemic antibiotic therapy was required for overt secondary infection of the skin, if they had received topical or systemic antibiotics for any reason during the preceding 4 weeks, or if potent topical steroids (British National Formulary category 1) were needed to control disease activity. All patients or their parents gave informed consent and the study was approved by the ethical committees of both hospitals at which patients were studied.

Forty-nine patients were entered into a double-blind placebo-controlled cross-over study and were randomly allocated to receive placebo first (P/M group) or mupirocin first (M/P group). Patient details are given in Table 1. At the initial visit 2 weeks before the start of the trial all patients were given a standard topical steroid therapy (0·5% clobetasol butyrate ointment, Eumovate) which was continued throughout the trial. Patients were asked to apply the topical steroid twice daily during the 2 week pre-trial period and once daily while using mupirocin or placebo. Patients were re-assessed clinically, by the same observer, at 2-week intervals for 10 weeks. For the first two weeks of the trial (weeks 0–2) patients in the M/P group applied

TABLE 1. Details of patients who received placebo followed by mupirocin (P/M group) and mupirocin followed by placebo (M/P group)

		P/M group ($n=21$)	M/P group ($n=24$)
Sex		12M 9F	12M 12F
Age:	Mean	20·4	22·4
(years)	(and range)	(2–52)	(2–56)
Race:	Caucasian	19	22
	Indian	2	1
	Chinese	0	1
Other atopic disease:			
	Asthma	8	10
	Hay fever	6	4
	None	7	10

mupirocin ointment and patients in the P/M group applied the polyethylene glycol ointment base (placebo). The trial ointment was applied in the morning and the topical steroid at night to all affected sites. After 2 weeks the groups were crossed over and the P/M group received mupirocin and the M/P group received placebo for a further 2 weeks (weeks 2–4). All the patients used clobetasol butyrate only twice daily for the remaining 4 weeks of the study period. The only other topical preparations allowed were the patients' usual emollients which were continued unchanged throughout the trial.

Patients were assessed clinically on a 0–3 scale for six features (extent, erythema, pustulation, excoriations, dryness and cracking) at 16 body sites. An estimate of the percentage body surface area involved was made using the rule of nines, which estimates the body surface area using nine or multiples of it, i.e. arms and head 9% each, anterior trunk, posterior trunk and legs 18% each.

Each patient was scored by the same observer at each visit to avoid inter-observer variation. Observers were blind as to the topical preparation used and the bacteriological findings. Patients were also asked to make a subjective assessment on a five point scale (much better, better, same, worse, much worse) of itch, their sleep disturbance and the appearance of their skin.

Bacteriological assessment. Bacteriological results were scored blind by the same bacteriologist at weeks 0, 2, 4, 6 and 8 on samples taken from two skin sites on each patient, the site 'worst affected' by atopic dermatitis and a 'control' site—an area of clinically normal skin unaffected by eczema.

TABLE 2. Bacterial isolates from the worse affected skin sites at week 0

		Number of patients with colony counts of *S. aureus*	
Isolates*	Total	$<10^2$	$\geqslant 10^2$
M/P group			
S. aureus	20	2	18
Coagulase negative staphylococcus	1		
Clostridium perfringens	1		
Acinetobacter spp.	1		
P/M group			
S. aureus	18	2	16
Coagulase negative staphylococcus	1		
β-haemolytic streptococcus (Lancefield Group G)	2		
Clostridium perfringens	2		

*Cultures from two patients in the M/P group and four patients in the P/M group grew two organisms, while cultures from three patients in the M/P group and two in the P/M group showed no growth.

Quantitative assessment of S. aureus

Numbers of *S. aureus* were estimated by applying 55 mm diameter contact plates (Sterilin Ltd. Middlesex, U.K.) directly to the skin sites. The plates contained cysteine, lactose, electrolyte deficient (CLED) agar (Oxoid Ltd, U.K.) which gives good colony differentiation of *S. aureus* and prevents the swarming of *Proteus spp*.

After aerobic overnight incubation at 37°C, the number of colonies of *S. aureus* per contact plate, i.e. per 24 cm^2, was counted, which represented the number of viable colony-forming units of *S. aureus* in the area of skin sampled.

Qualitative assessment

Swabs (Albuswabs, Exogen, Clydebank, U.K.) were taken from worst affected and control sites. Each swab was used to inoculate blood agar, blood agar with gentamicin (10 mg/l) MacConkey agar and Robertson's meat broth. One blood agar plate was incubated aerobically and one anaerobically and the remaining media were incubated aerobically at 37°C overnight.

The blood agar, with and without gentamicin, was important for detecting β-haemolytic streptococci, which are less easily identified on CLED agar.

Swabs taken from the patients' anterior nares at week 0 were plated on blood agar and incubated aerobically at 37°C overnight.

Organisms were identified by routine laboratory methods. Sensitivity to commonly used antibiotics was tested by the comparative disc diffusion method.[14] Minimum inhibitory concentrations (MICs) of mupirocin for all *S. aureus* isolates were determined by the broth dilution method,[15] and phage typing was carried out according to standard methods.

RESULTS

Four patients were excluded from the final analysis. Three had received placebo first (P/M group) and one mupirocin (M/P group). One patient in the M/P group had an acute flare requiring potent steroids during the run-in period, a second, in the P/M group, required concomitant antibiotics for a respiratory infection and a third, in the P/M group, reacted adversely to both mupirocin and placebo. Only one patient defaulted while on mupirocin. Thus, 45 patients were available for analysis.

TABLE 3. Numbers of patients colonized with *S. aureus* (i.e. colony counts $\geqslant 10^2$)

	Worst affected sites					Control sites				
	Week 0	Week 2	Week 4	Week 6	Week 8	Week 0	Week 2	Week 4	Week 6	Week 8
	End of run-in	End of mupirocin	End of placebo			End of run-in	End of mupirocin	End of placebo		
M/P group	18 of 24	1 of 24	7 of 21	13 of 20	9 of 20	4 of 24	0 of 24	2 of 21	2 of 20	9 of 20
	End of run-in	End of placebo	End of mupirocin			End of run-in	End of placebo	End of mupirocin		
P/M group	16 of 21	14 of 21	1 of 20	9 of 19	8 of 18	2 of 21	4 of 21	1 of 20	2 of 19	2 of 18

Forty-six isolates were obtained from the worst affected sites of the 45 patients at week 0. *S. aureus* was isolated from 38 of the 45 patients; five patients carried two strains and one carried three strains giving a total of 45 strains. β-haemolytic streptococci of Lancefield Group G were isolated on two occasions. No β-haemolytic streptococci of Lancefield Group A were isolated. An *Acinetobacter sp*, a heavy growth of coagulase negative staphylococci, and *Clostridium perfringens* were isolated on one, two and three occasions, respectively (Table 2). Small numbers of coagulase negative staphylococci and diphtheroids in mixed culture were regarded as normal flora.

Control non-affected sites yielded similar results. Twenty-five positive isolates were obtained at week 0 of which 23 were *S. aureus*. *Clostridium perfringens* and a heavy growth of coagulase negative staphylococci were each isolated on one occasion. Small numbers of coagulase negative staphylococci and diphtheroids in mixed culture were again regarded as normal flora.

With the exception of one isolate, all the *S. aureus* isolates were sensitive to mupirocin with MICs between 0·125 and 0·5 mg/l. The more resistant isolate (MIC 32 mg/l) was isolated from the worst affected site of a patient in the M/P group 4 weeks after stopping mupirocin. No methicillin resistant strains of *S. aureus* were isolated.

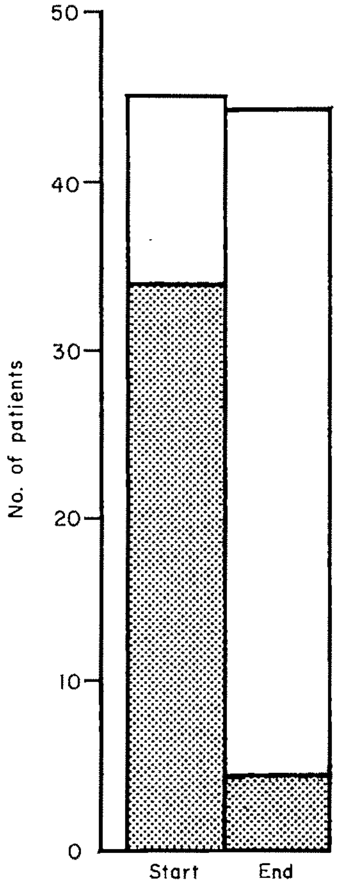

FIGURE 1. Numbers of patients colonized by staphylococci before and after mupirocin treatment (M/P and P/M groups combined) at worst affected sites. ▨ ≥ 100 colonies/24cm²; □ < 100 colonies/24 cm².

TABLE 4. Phage typing of 45 strains of *S. aureus* from the worst affected area at week 0

Phage type	No of strains (%)	
Group III	14	(31%)
Group I	4	(9%)
Group II	3	(7%)
Other	9	(20%)
Non-typable	15	(33%)

Full phage type details of all strains available on request.

S.aureus colonization

The colony counts of *S. aureus* per 24 cm^2 (contact plate surface area) were arbitrarily divided into two mutually exclusive and exhaustive categories: counts $\geqslant 10^2$, designated 'colonized', and counts $< 10^2$ including 0 (Tables 2 and 3). Differences in the number of colonized sites between the patient groups, and within patient groups at different times, were analysed using Fisher's exact test.

At week 0, 6 of the 45 patients (13%) were colonized at the 'control' clinically normal site while 39 patients (87%) carried less than 10^2 colonies and 20 patients (44%) did not carry any *S. aureus* at these sites.

On the 'worst affected' sites, at week 0, 34 of the 45 patients (76%) were colonized while 11

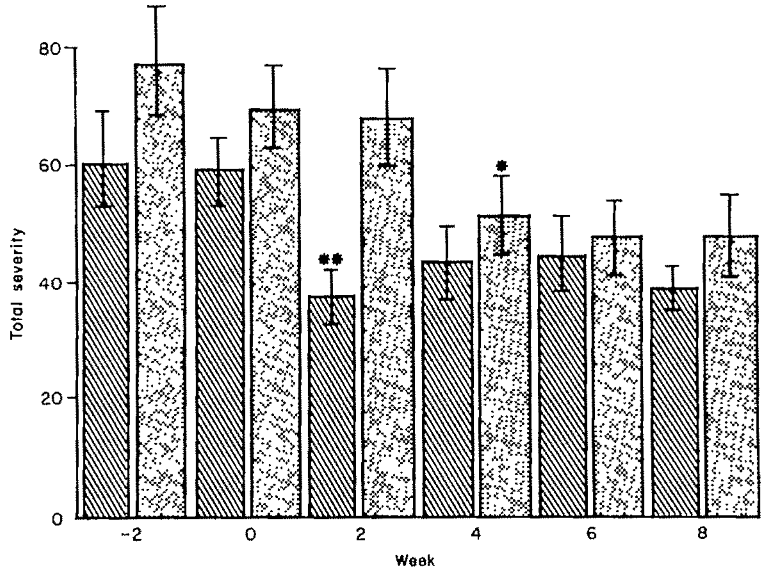

FIGURE 2. Clinical severity scores in the M/P group (■) and P/M group (▨). Bars are means ± SEM. After treatment with mupirocin, clinical activity fell and was significantly different from week 0 $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}001$.

patients (24%) carried less than 10^2 colonies. In only 7 of the 45 patients (16%) was no *S. aureus* isolated from the worst affected site at week 0.

There was no significant difference between the number of sites colonized in the two groups, either 'worst affected' or control, at week 0, but in both groups the number of worst affected sites colonized was significantly greater than the number of control sites ($P < 0{\cdot}0001$). In the P/M group there was no significant difference between the number of sites colonized at week 0 and week 2, i.e. before and after application of the placebo ($P > 0{\cdot}05$). Thus there was no evidence that that the polyethylene glycol ointment base had anti-staphylococcal activity.

In contrast, there was a significant fall in the number of worst affected sites colonised in both groups after 2 weeks of mupirocin therapy ($P < 0{\cdot}0001$). *S. aureus* could be recovered from only 4 of 44 patients (9%) at this time (Fig. 1).

Recolonization to pre-treatment levels was found at week 6, 2 weeks after the end of mupirocin treatment in the P/M group and 4 weeks after the end of mupirocin treatment in the M/P group. At this time the number of colonized sites in each group did not differ significantly from that before treatment ($P > 0{\cdot}05$).

Phage typing was carried out on all strains of *S. aureus*. The results for the 45 strains isolated from the worst affected site at week 0 are shown in Table 4. The majority belonged to group III; there were three from group II and four from group I. At week 0, 12 of the 45 patients (27%) carried the same phage type in the nose as that in the worst affected area and 14 of the 45 (31%) had the same phage type in the worst affected and the control sites. In only eight patients (18%) was the same phage type present in all three sites.

Over the period of the study 22 of the 45 patients (49%) were found to carry more than one strain of *S. aureus*. After treatment with mupirocin 12 of the 45 (27%) were recolonized with the same strain as that originally found in the nares, 14 of 45 (31%) with that from the worst affected area and 9 of 45 (20%) with that from the control site. Eight patients were recolonized with a 'new' strain that had not previously been isolated.

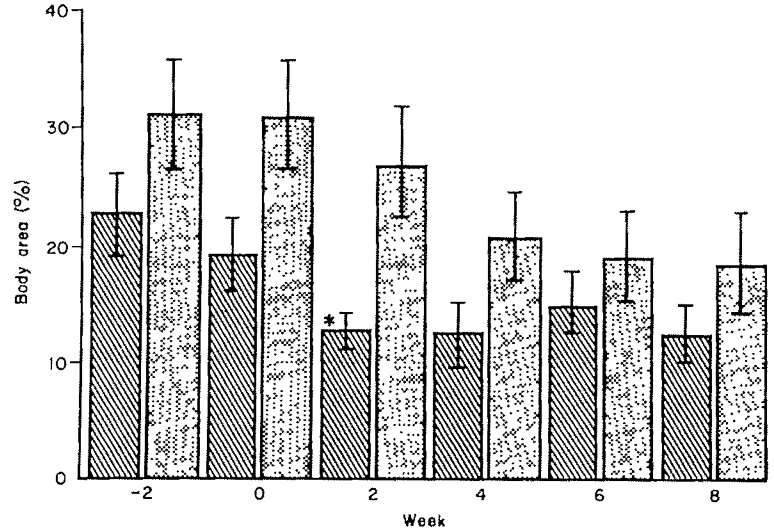

FIGURE 3. Percentage body area affected in M/P group (■) and P/M group (▨). Bars are means ± SEM. * Significantly different from week 0, $P < 0{\cdot}05$.

TABLE 5. Patients' assessments of skin appearance, itch and sleep disturbance

	Weeks 0–2 M/P group No. of patients					Weeks 0–2 P/M group No. of patients					Weeks 2–4 P/M group No. of patients				
	Better	Same	Worse	$\chi^{2\dagger}$	P	Better	Same	Worse	$\chi^{2\dagger}$	P	Better	Same	Worse	$\chi^{2\dagger}$	P
Skin appearance	19**	5	0	19·0	<0·001	10	8	3	3·8	NS	8	10	3	2·3	NS
Itch	13**	10	1	10·3	<0·001	4	13	4	0	NS	9	10	2	4·5	NS
Sleep disturbance*	12**	10	0	12·0	<0·001	3	13	3	0	NS	2	14	3	0·2	NS

*Two patients in each group had no sleep disturbance.

**A significant proportion of patients showed an improvement ($P < 0{\cdot}05$; Fishers exact test).

† χ^2: within group test of differential change. All have 1 degree of freedom.

Clinical pre-treatment severity scores showed that the P/M group was slightly more severely affected, mean score ± SEM 69·9 ± 6·9 than the M/P group, mean score 59·5 ± 6·4 and also had a greater mean surface area involved, 31·0 ± 4·7% versus 19·1 ± 3·0% in the M/P group. After the 2-week run-in period, clinical severity fell in the P/M group, but remained virtually unchanged in the M/P group. After the first trial period, clinical severity was virtually unchanged in the P/M group (using placebo) mean score 68·0, but fell significantly in the M/P group (using mupirocin) to a mean of 37·6. This was significantly different both from the M/P group pre-treatment baseline ($P < 0{\cdot}001$) and from the P/M group ($P < 0{\cdot}002$). After the cross-over period at 4 weeks, the P/M group improved significantly ($P < 0{\cdot}05$) whereas the M/P group, (now on placebo) showed a slight, but not statistically significant deterioration. Clinical activity then remained stable in both groups for the remainder of the trial (Fig. 2). Calculation of the change in clinical severity scores over the whole trial period taking visit 0 as the base-line value, showed that the change in clinical severity was virtually identical for the two groups.

Similar, though less marked changes were seen in the area involved; the P/M group showed a diminution in surface area involved during both the run-in period and the placebo phase although a more marked fall was seen when mupirocin was applied. However, this was not clinically significant. In contrast, the area involved in the M/P group was unchanged during the run-in period, but fell significantly when the active treatment was applied ($P < 0{\cdot}003$). In both groups the area involved remained unchanged for the rest of the trial (Fig. 3).

On subjective assessment the patients felt they had improved symptomatically in terms of skin appearance, itch and sleep disturbance while they were using topical mupirocin. However, this was only statistically significant in the group which received mupirocin first ($P < 0{\cdot}001$; Fisher's Exact test) (Table 5).

No serious side-effects were observed. Two patients noticed that both the placebo and the mupirocin ointments caused stinging and an exacerbation of their symptoms. This caused one patient to withdraw from the trial.

DISCUSSION

The present study has confirmed that both eczematous and unaffected areas of skin are colonized with *S. aureus* in patients with atopic dermatitis.[1,3,4] However, the normal areas of skin may well be colonized by the simple transfer of organisms from the affected eczematous areas. It is difficult to compare these present quantitative studies directly with previously reported scrub techniques for sample collecting.[3,4] The plating method has the advantage that it does not irritate eczematous skin and is quick and painless, which is an important consideration in investigating children.

The clinical improvement following mupirocin was associated with a reduction in staphylococcal colonization which raises the question of the role of *S. aureus* in this disease.[16] Recolonization did not appear to be associated with clinical deterioration within the period of the study. This does not exclude a pathogenetic role for *S. aureus*, but their effect, if present, must be delayed.

Phage typing in general confirmed the finding by Aly[2] that most of the staphylococci belong to, or cross react with, group III. However, group I (hospital) strains were isolated from three of our patients and three strains were from group II which are associated with the 'staphylococcal scalded skin syndrome'. We also found that many of our patients with atopic dermatitis carried more than one strain of staphlyococcus in different sites, which contrasts with the findings of Anderson[17] who noted that different regions of the same individual were generally colonized with the same phage type. He also showed that swabs taken from seven patients at different times (range 1 to 6 weeks) were generally colonized with the same organism. This was not the pattern we found. However, our results represented recolonization after topical antibiotic treatment, which may differ from that found in the 'normal' flora of a patient with atopic dermatitis which has not been altered by treatment.

Mupirocin, but not the polyethylene glycol (PEG) base significantly reduced this staphylococcal colonization, and in 89% of patients, staphylococci were not isolated from the skin after 2 weeks application of mupirocin. Other studies have shown similar reduction of staphylococcal colonization using topical neomycin and systemic erythromycin.[3] During mupirocin application, clinical activity also fell significantly. After mupirocin was discontinued, recolonization occurred fairly rapidly, but a concomitant clinical deterioration was not observed. Superinfection with other organisms was not seen during the trial, nor was this observed by Lin *et al.*[18] who used mupirocin in the treatment of epidermolysis bullosa over a period of 2 years. Recently, however, there have been reports of the development of resistant strains[19,20] and resistance may be transferable by plasmids.

A further observation in our study was that the replacement of the normal commensal lipophilic diphtheroids with *S. aureus* produced little sign of infection in atopic dermatitis. The effect of staphylococcal colonization, therefore, may simply be a direct inflammatory one, possibly induced by protein A in the cell wall.[21]

During the present study a reduction in the bacterial load was noted to be associated with clinical improvement, suggesting that control of skin colonization with staphylococci may prove clinically beneficial in the treatment of atopic dermatitis.

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In vivo effects of interferon-γ and anti-interferon-γ antibody on the experimentally induced lichenoid tissue reaction

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SUMMARY

We investigated the *in vivo* effect of recombinant interferon-γ (IFN-γ) and tumour necrosis factor α (TNF-α) treatment of mice on the development of the delayed-type hypersensitivity (DTH) reaction and lichenoid tissue reaction (LTR) following the local injection of cloned autoreactive T cells. Both the DTH reaction and the LTR were significantly enhanced by pre-treatment with IFN-γ, but not with TNF-α. Induction of class II MHC antigens on keratinocytes was not essential for the enhancement by IFN-γ. Administration of anti-IFN-γ antibody reduced the DTH reaction and LTR, although complete inhibition was not observed with our treatment regimen. The ability of IFN-γ to increase the number of the cloned T cells invading the epidermis *in vivo*, is in keeping with our previous observation that IFN-γ treatment of cultured keratinocytes markedly increased the adherence reaction between T cells and keratinocytes *in vitro*.

Interferon-γ (IFN-γ) is a lymphokine produced by T cells upon activation by an antigen or mitogen. In addition to its antiviral and antiproliferative activities,[1] IFN-γ has a number of effects which favour the development of inflammation: it induces the expression of class II major histocompatibility complex (MHC) antigens on normally negative cells including keratinocytes,[2] enhances lymphocyte adhesion molecule expression by monocytes,[3] functions as a macrophage-activating factor,[4] and augments the phagocytic and cytotoxic activities of neutrophils.[5] Aberrant expression of class II MHC antigens on normally negative cells in a variety of autoimmune diseases[6] and the modulation of this expression by IFN-γ[2] raise the intriguing possibility that unregulated production of IFN-γ may be central to the development of autoimmunity.[7]

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We have recently demonstrated that murine autoreactive cloned T cells capable of producing IFN-γ and tumour necrosis factor β (TNF-β) evoke the cutaneous delayed-type hypersensitivity (DTH) reaction and lichenoid tissue reaction (LTR) upon intradermal inoculation into normal syngeneic hosts.[8] The LTR evoked by these T cells was most probably mediated by a synergistic effect between IFN-γ and TNF-β released from the T cells in situ.[9,10] Since IFN-γ has been observed to greatly enhance the adherence reaction between T cells and cultured keratinocytes *in vitro*,[11] we investigated the possibility of an *in vivo* counterpart of this reaction. These findings led us to investigate the *in vivo* effect of recombinant IFN-γ and anti-IFN-γ antibody on the development of the lichenoid tissue reaction.

METHODS

Animals

Female C57BL/6 (B6) mice were obtained from Charles River Japan, Inc. and maintained in our animal facility. They were used predominantly at 8 to 12 weeks of age.

Induction of the lichenoid tissue reaction

Two different kinds of autoreactive cloned T cells specific for class II MHC antigens, I-A^b, were established in previous experiments.[9,10] BB5 cells, when injected into the footpads of syngeneic B6 mice, migrate into the epidermis and cause the destruction of the epidermal cells.[8] C10 cells, in contrast, are completely incapable of migrating into the epidermis and consequently fail to induce the epidermal change.[8]

Assay for the delayed type hypersensitivity reaction

The cloned T cells were harvested from culture 7–8 days after antigenic stimulation. The T cells with a viability greater than 95% were injected intradermally, at different doses and in a volume of 25 μl, into the hind footpads of B6 mice. The resultant swelling of the footpads was measured with a dial thickness gauge 48 h after injection, as described previously.[8]

Evaluation of the lichenoid tissue reaction

At 72 h after injection of the T cells, skin samples from the hind footpads of the mice were excised using scissors, fixed in 3·7% buffered formaldehyde, embedded in paraffin, sectioned, and stained with haematoxylin and eosin. The LTR was defined as the intensity of epidermal invasion by the injected T cells as follows. A semi-quantitative evaluation of epidermal invasion by the injected T cells was performed by counting lymphoid cells within the epidermis. At least 30 serial sections from each individual footpad were examined, and of these the six sections in which the epidermis was regarded to be most heavily infiltrated with lymphoid cells were counted. The results were expressed as the number of lymphoid cells per linear mm of epidermis, as previously described.[8]

Reagents

Recombinant mouse IFN-γ (0·98 mg/ml; specific activity: 23×10^6 U/ml), purified rabbit antibody against mouse IFN-γ (1·2 mg/ml; 1 ml of antibody neutralizes $1{\cdot}28 \times 10^5$ U of IFN-γ) and recombinant human TNF-α (0·50 mg/ml; specific activity: $2{\cdot}51 \times 10^7$ U/ml) were a gift from Dr. H.M.Shepard (Genetech, Inc., South San Francisco, CA, U.S.A.). They were stored at 4°C and diluted in phosphate-buffered saline (PBS) before use. IFN-γ or TNF-α was injected intradermally into the hind footpads of B6 mice on 2 or 6 successive days at a dose of $0{\cdot}5$–5×10^4

U/day. Control mice received the corresponding quantities of PBS alone. In some experiments, on days 0 and 1 or 1 and 2, anti-IFN-γ antibody (50 μl) was injected into the same footpad site that had received BB5 cells on day 0. In these experiments, control mice received 50 μl PBS containing 60 μg of chromatographically purified rabbit IgG (Cappel Laboratories, Cochville, PA, U.S.A.). Because no significant changes were observed between mice injected with PBS alone and those injected with rabbit IgG-containing PBS, with respect to footpad swelling and histology, the latter mice were used as the proper controls in these experiments.

RESULTS

Local administration of IFN-γ enhanced the DTH reaction and lichenoid tissue reaction induced by the autoreactive T cells

To elucidate the effect of IFN-γ *in-vivo* on the DTH reaction and LTR induced by the local transfer of the autoreactive T cells, groups of normal B6 mice were pre-treated by the intradermal injection with $0{\cdot}5 \times 10^4$ U of either IFN-γ or TNF-α on the two days preceeding injection of the T cells. This dose of IFN-γ or TNF-α was selected because in the absence of added autoreactive T cells, it has been found not to evoke significant pathological changes. On day 0, 1×10^6 BB5 cells were injected into the same site that had been injected with IFN-γ, TNF-α or PBS. As shown in Table 1 and Fig. 1, pre-treatment with IFN-γ significantly enhanced the epidermal invasion by BB5 cells and, to a lesser extent, the DTH reaction, while the effect of TNF-α *in vivo* was slight. When BB5 cells were given at a lower dose ($0{\cdot}5 \times 10^6$ cells/mouse), the enhancement was more pronounced. Moreover, concomitant injection of anti-IFN-γ antibody with IFN-γ abrogated its ability to enhance the DTH reaction and LTR induced by BB5 cells. However, when IFN-γ was injected after the injection of BB5 cells, the enhancement was found to be minimal. Compared with treatment with IFN-γ alone, no augmentation was observed after simultaneous treatment with IFN-γ and TNF-α. We then

TABLE 1. Ability of IFN-γ to enhance the DTH reaction and LTR

No. of T cells injected	Treatment		Increase in footpad thickness (mm × 10^{-2}) mean ± SD	Lymphoid cells invading the epidermis (cells/mm) mean ± SD
	Agent	Injection on day		
Experiment 1				
1×10^6 BB5	PBS	−2, −1	32·5 ± 2·3	95 ± 6
1×10^6 BB5	IFN-γ	−2, −1	37·8 ± 1·9*	171 ± 9**
1×10^6 BB5	TNF-α	−2, −1	36·8 ± 2·9	108 ± 11
1×10^6 C10	PBS	−2, −1	16·3 ± 1·2	0
1×10^6 C10	IFN-γ	−2, −1	20·7 ± 1·7*	0
Experiment 2				
$0{\cdot}5 \times 10^6$ BB5	PBS	−2, −1	21·0 ± 2·7	24 ± 3
$0{\cdot}5 \times 10^6$ BB5	IFN-γ	−2, −1	26·3 ± 2·4*	51 ± 9**
$0{\cdot}5 \times 10^6$ BB5	IFN-γ + anti-IFN-γ	−2, −1	18·8 ± 1·9	20 ± 3
$0{\cdot}5 \times 10^6$ BB5	IFN-γ + TNF-α	−2, −1	25·8 ± 1·8*	55 ± 6**
$0{\cdot}5 \times 10^6$ BB5	IFN-γ	1,2	19·8 ± 2·1	28 ± 2*

Values are means ± SD of 3–4 mice.
Significantly different from control; $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}005$ (Student's *t*-test).

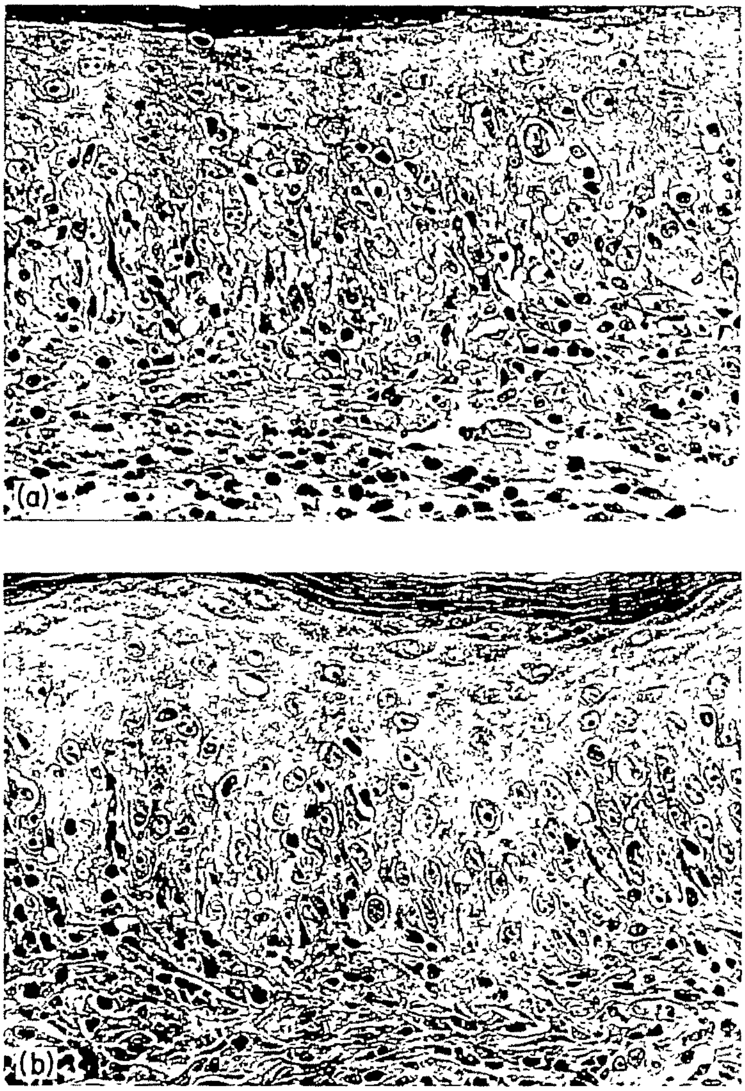

FIGURE 1. Epidermal invasion of BB5 cells 72 h after injection of 1 × 10^6 cells into the footpads of syngeneic mice who had been injected at the same site on the 2 preceeding days with (a) IFN-γ 0·5 × 10^4U or (b) PBS. (H&E; original × 120).

investigated whether local administration of IFN-γ could induce a non-epidermotropic clone, C10, to migrate into the epidermis. In contrast to the enhanced migration of BB5 cells, C10 cells were still incapable of migrating into the epidermis after *in vivo* treatment with IFN-γ, although the DTH reaction was similarly enhanced.

The ability of IFN-γ to enhance the DTH responses and LTR is not mediated through the induction of I-A antigens by keratinocytes

It has been suggested that the expression of I-A (class II MHC antigens in mice) by keratinocytes may help direct the migration of lymphocytes into the skin during DTH

TABLE 2. Effect of multiple IFN-γ administration on the DTH reaction and LTR

No. of BB5 cells injected	Treatment		Increase in footpad thickness ($mm \times 10^{-2}$) mean ± SD	Lymphoid cells invading the epidermis (cells/mm) mean ± SD
	Agent	Injection on day		
1×10^6	PBS	−6, −5, −4, −3, −2, −1	28·8 ± 1·1	53 ± 7
1×10^6	IFN-γ (5×10^4U)	−6, −5, −4, −3, −2, −1	37·0 ± 2·9*	185 ± 13**

Values are means ± SD of 4 mice.
Significantly different from control; $*P < 0{\cdot}05$, $**P < 0{\cdot}005$ (Student's *t*-test).

responses.[12] We, therefore, tested whether the ability of IFN-γ to induce keratinocytes to express I-A antigens was involved in the observed enhancement of the DTH reaction and LTR by IFN-γ. However, after *in vivo* treatment with $0{\cdot}5 \times 10^4$ U IFN-γ for 2 days, the epidermis did not show intercellular staining for I-A antigens, as judged by indirect immunofluorescence staining of frozen sections of footpad skin. The induction of I-A antigens on keratinocytes was found to be obtained with 5×10^4 U IFN-γ given on 6 consecutive days (data not shown). The repeated intradermal injections with this dose of IFN-γ were, however, usually associated with a significant increase in dermal infiltrates, that were not observed in mice injected with $0{\cdot}5 \times 10^4$ U IFN-γ on 2 consecutive days. However, no intra-epidermal lymphoid cells were observed in mice that had received 5×10^4 IFN-γ for 6 days, but not followed by the injection of BB5 cells. As shown in Table 2, 6 days of treatment with higher doses (5×10^4 U) of IFN-γ markedly enhanced the DTH reaction and LTR. These results suggest that the enhancement by IFN-γ could be dose-dependent, although we cannot exclude the possibility that the higher dose of IFN-γ would have resulted in endogenous IFN-γ production by host lymphocytes, thus contributing to some of the observed enhancement.

Treatment with anti-IFN-γ antibody reduced the DTH reaction and LTR

In order to test whether anti-IFN-γ antibody could prevent the development of the LTR, mice that had received 1×10^6 BB5 cells were given intradermal injections with anti-IFN-γ antibody (50 μl). Preliminary studies indicated that treatment of normal mice with this dose of anti-IFN-γ antibody elicited neither significant dermal infiltration, nor footpad swelling. As shown in Table 3, administration of anti-IFN-γ antibody significantly reduced the DTH reaction and epidermal invasion by BB5 cells when the administration was delayed to day 1 after injection of BB5 cells. In contrast, prevention of the DTH reaction and LTR by anti-IFN-γ antibody was not observed when mice were injected with the antibody immediately after the injection of BB5 cells. The degree of inhibition by anti-IFN-γ antibody was more profound in the DTH reaction than in the LTR. However, two intradermal injections of anti-IFN-γ antibody at 24 h intervals did not completely abrogate the capacity of BB5 cells to elicit the DTH reaction and LTR.

DISCUSSION

To clarify the role of IFN-γ in the pathogenesis of the lichenoid tissue reaction, we treated mice with repeated injections of recombinant mouse IFN-γ before the local injection of cloned T cells capable of inducing the LTR. The treatment enhanced the DTH reaction and the LTR induced by the T cells. This enhancement appeared not to be due to the induction of I-A antigens on

TABLE 3. Effect of anti-IFN-γ antibody on the DTH reaction and LTR

No. of BB5 cells injected	Treatment		Increase in footpad thickness $(\text{mm} \times 10^{-2})$ mean ± SD	Lymphoid cells invading the epidermis (cells/mm) mean ± SD
	Agent	Injection on day		
Experiment 1				
1×10^6	PBS	0	30·3 ± 3·8	95 ± 6
1×10^6	anti-IFN-γ	0	33·3 ± 2·2	102 ± 16
1×10^6	PBS	1	32·5 ± 2·3	99 ± 8
1×10^6	anti-IFN-γ	1	22·8 ± 2·4**	80 ± 10*
Experiment 2				
1×10^6	PBS	1	35·2 ± 3·1	81 ± 10
1×10^6	anti-IFN-γ	1	27·5 ± 2·1*	72 ± 6
1×10^6	PBS	1,2	ND	89 ± 7
1×10^6	anti-IFN-γ	1,2	ND	62 ± 7**

Values are means ± SD of 3–4 mice.
Significantly different from control; $^{*}P < 0{\cdot}01$, $^{**}P < 0{\cdot}005$ (Student's t-test)
ND = not done.

keratinocytes by IFN-γ, because I-A antigen expression on keratinocytes was never observed in footpads injected with the lower dose of IFN-γ on 2 consecutive days, where both the DTH reaction and LTR were significantly enhanced. It has been proposed that in the LTR, IFN-γ secreted by activated lymphocytes in the upper dermis could induce basal keratinocytes to express class II MHC antigens in a wide variety of dermatoses[13] and that I-A antigen expression by keratinocytes may permit them to function as antigen-presenting cells, like Langerhans cells.[14] However, experimental evidence has recently been presented to challenge such a hypothesis. Gaspari *et al.*[15] have shown that class II antigen positive keratinocytes do not replace Langerhans cells in their capacity to induce various immune responses. Our present finding also argues against the critical role of class II antigen positive keratinocytes in the induction of the LTR, although our failure to detect I-A antigens on keratinocytes after treatment with the lower dose of IFN-γ does not necessarily exclude their presence, or their appearance later and possible significance in perpetuating the inflammatory response.

What, then, is the mechanism by which IFN-γ enhances the DTH reaction and LTR? IFN-γ has been shown to induce synthesis of TNF receptors in some cells and thereby increase their sensitivity to the cytostatic activity of TNF.[16] It is, therefore, possible that exogenous IFN-γ may act to increase TNF receptors on keratinocytes, thereby rendering them more sensitive to TNF released from activated T cells and macrophages. In addition, IFN-γ preferentially enhances the interleukin 1 (IL-1) secretory potential of monocytes.[17] Because epidermal cell-derived thymocyte-activating factor (ETAF)/IL-1 has been reported to be a potent chemoattractant for T cells,[18] IFN-γ may act to enhance the migration of T cells into the epidermis, through its capacity to stimulate keratinocytes to release ETAF.

With respect to a role for IFN-γ in promoting T cell adhesion to keratinocytes, we have recently observed that IFN-γ-treated keratinocytes display a markedly increased adherence reaction towards monocytes and T lymphocytes.[11,19] This adherence reaction did not appear to involve class II antigens, as antibody to this antigen did not inhibit the reaction, but antibody against lymphocyte function-associated antigen 1 (LFA-1) totally blocked the binding. Thus, it is possible that IFN-γ increased the number of BB5 cells in the epidermis by promoting the adherence reaction between lymphocytes and keratinocytes *in vivo* as we have previously observed *in vitro*. In fact, the kinetics of the reaction *in vivo* agree well with our *in vitro* observations[19] in which significant enhancement of binding by IFN-γ was seen between 1 and 2 days. When we compared IFN-γ to TNF-α in the *in vitro* adherence assay, IFN-γ was better than TNF, and the failure to observe significant changes with TNF-α in the present *in vivo* study may reflect the requirement for higher concentrations of TNF-α to be used than could be achieved in the present study.

It has been shown that TNF-α and -β, secreted by macrophages and lymphocytes, respectively, share several activities with IFN-γ, including cytotoxic or cytostatic effects[20] and ability to enhance the expression of a family of leukocyte adhesion molecules.[21] Moreover, the synergistic effect of the two lymphokines is frequently observed[22] and most IFN-γ-producing T cells as well as our cloned T cells can also release TNF-β (N.H. Ruddle, personal communication).[23] It is possible, therefore, that the two lymphokines could exist in combination, but not in isolation, in the physiological environment. However, the apparent incapacity of TNF-α to enhance the LTR and to act synergistically with IFN-γ suggests that the enhancement of the LTR may be a property unique to IFN-γ. Alternatively, because TNF treatment has been reported to result in synthesis of prostaglandins,[24] the capacity of TNF-α to enhance the DTH reaction and the LTR may have been abrogated through the generation of prostaglandins, which has been shown to suppress cell-mediated immune responses including DTH reactions.

In the light of the crucial role of IFN-γ in the pathogenesis of the LTR, administration of anti-IFN-γ antibody might constitute an effective strategy for the treatment of various lichenoid skin diseases. Our results show that although partial prevention of the LTR was achieved by treatment with anti-IFN-γ antibody, the reduction was less pronounced than expected from the *in vivo* enhancing effect of IFN-γ. The reason why the DTH reaction and LTR were not completely abrogated by anti-IFN-γ antibody treatment is not clear, but might have simply been due to use of anti-IFN-γ antibody at insufficient concentrations, or failure to maintain effective concentrations for a sufficiently long period to neutralize IFN-γ released from activated T cells in situ. Thus, it remains to be determined whether treatment with anti-IFN-γ antibody could be a useful therapeutic approach to LTR and other autoimmune diseases, as is the case with antibodies to T cell surface molecules.[25]

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Immunomodulation and Sézary syndrome: experience with thymopentin (TP-5)

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SUMMARY

The clinical, histological and immunological effects of long-term treatment with thymopentin (TP-5), administered 50 mg i.v. three times a week on alternate days, in four patients with Sézary syndrome is reported.

In all four cases reduction of itching, oedema, scaling and thickening, and clearing of erythroderma were noted after 2 months treatment. Peripheral blood Sézary cells decreased in three cases. Reduction or suspension of the drug was followed by a clinical relapse.

A loss of epidermotropism and a reduction in cell infiltrates were observed together with a dramatic reduction in epidermal and dermal Langerhans cells.

An increase in the proliferative response to mitogens and in IFN-γ production, and the expression of activation antigens in PHA stimulated cultures occurred after 3 months. HNK-1+ cells increased both in the peripheral blood and in the dermis following a transient increase in IL-2 receptors, suggesting that clinical response in TP-5 treated patients may be mediated by an increased production of IL-2 and consequent generation of cytotoxic cells or release of lymphokines able to augment NK activity.

A defective or absent proliferative response to mitogens by peripheral blood mononuclear cells (PBMC) has been observed in most patients with Sézary syndrome, particularly in its advanced stages.[1–4] Although atypical antigen patterns have been observed, most Sézary cells (SC) express the phenotype of mature helper T cells CD5+ CD4+ CD8− CD3+ CD2+. The CD3+ CD8+ sub-population is consistently reduced to 1–10% of PBMC in most patients, but no functional data on this cell type have been reported to our knowledge.

Since i.v. thymopentin (TP-5) has been shown to increase the numbers of CD8+ cells in patients with rheumatoid arthritis,[5] we tested this drug in four patients with Sézary syndrome to determine whether it improved the clinical status of the patients, whether the clinical response, if any, was accompanied by worthwhile histological and immunological skin response, and whether phenotypic or functional modifications of PBMC were induced.

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CASE REPORTS

Four patients with Sézary syndrome were initially infused with TP-5, 50 mg, i.v. in 10 min, three times a week on alternate days for 8–12 weeks. The dose was then modified according to the clinical response.

Case 1

A 54-year-old male, who had had Sézary syndrome since 1982 had been treated with leukapheresis, and prednisone plus low doses of chlorambucil and mechlorethamine for more than 3 years. He had had a generalized herpes infection with keratitis since the onset of the disease; this had shown transient remissions with acyclovir treatment. His erythroderma progressively worsened with an increase in skin thickness and oedema, 'facies leonina' (Fig. 1a), fissuring of the skin of the palms and soles (Fig. 2a), generalized loss of body hair and nail dystrophy (Fig. 3a). Two nodular lesions also appeared on the left forearm, in September 1985, together with an increase in lymphadenopathy. The Karnowsky performance status was 30%. The clinical picture was unchanged by VP-16-213 (etoposide) and mechlorethamine.

TP-5 was begun in October 1985. Immediately before TP-5 the WCC was $21{\cdot}2 \times 10^9$/l with 23% neutrophils, 35% eosinophils, 37% SC ($8{\cdot}029 \times 10^9$/l), 1% lymphocytes, and 4% monocytes. All laboratory tests were within the normal range, apart from elevated LDH (716 IU) and ESR (155 mm in the 1st h). The anti-HSV titre was 1 : 1024. Reduction of oedema, and scaling and thickening, clearing of erythroderma (Figs. 1b and 2b), regression of palmoplantar keratoderma and partial nail re-growth (Fig. 3b) were noted after 2 months treatment. Laboratory tests showed the normalization of LDH and the reduction of the ESR to 25 mm in

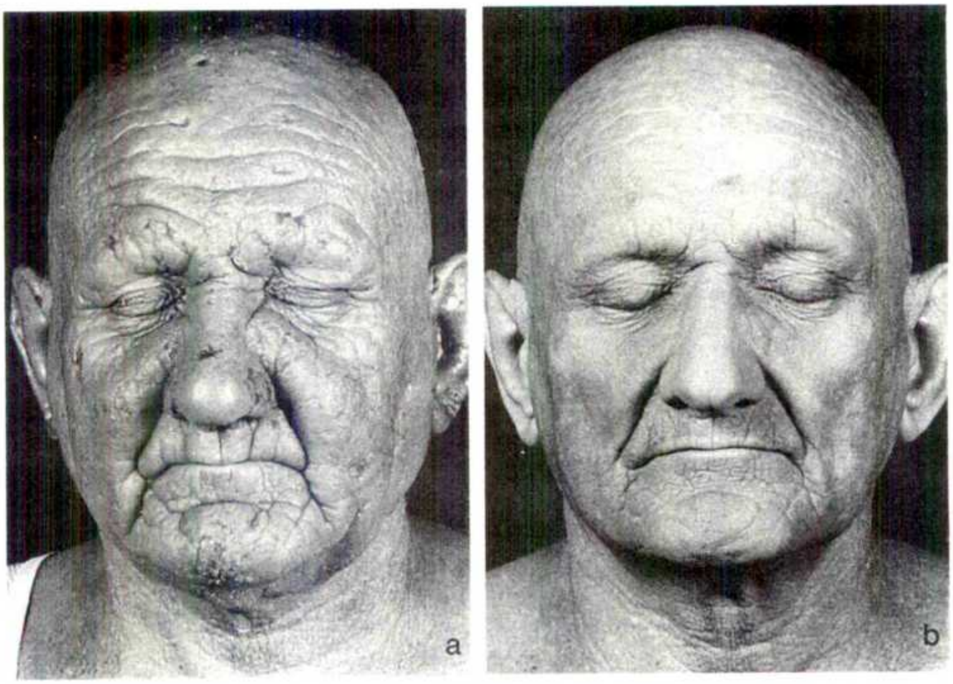

FIGURE 1. Case 1: (a) Before treatment and (b) after 7 months of TP-5 treatment.

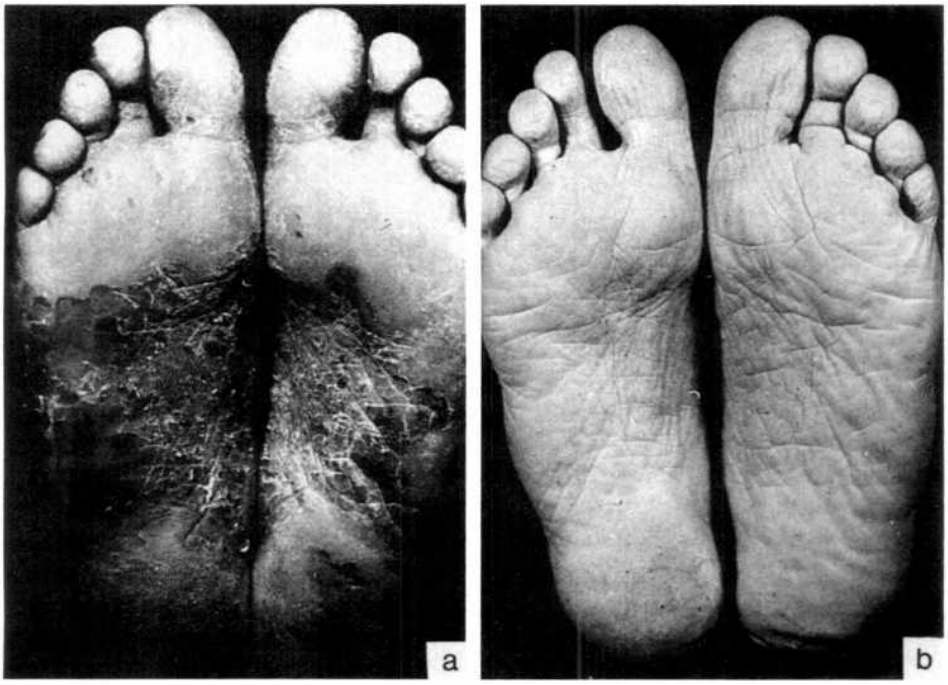

FIGURE 2. Case 1: (a) Before treatment and (b) after 7 months of therapy with TP-5.

the 1st h. The WCC was $6{\cdot}9 \times 10^9$/l with 76% neutrophils, 1% basophils, 8% eosinophils, 10% lymphocytes and 4% monocytes. Only a few SC (1%; $0{\cdot}7 \times 10^9$/l) were present in the peripheral blood and nearly all the lymphocytes presented an abundant cytoplasm with numerous azurophilic granules. The dose of TP-5 was then reduced to 50 mg per week for 5 months and 50 mg every 2 weeks for a further 2 months. The treatment was then suspended as the patient was in a clinical and histological remission. During this period the WCC ranged from 4·1 to $7{\cdot}2 \times 10^9$/l with lymphocytes ranging from 10 to 20%; SC never exceeded $0{\cdot}1 \times 10^9$/l. The treatment had to be recommenced at the original dose owing to relapse 1 month later. A further remission, in particular of the cutaneous symptoms and lymphadenopathy was obtained. At the time of writing, the patient was still receiving 50 mg three times per week.

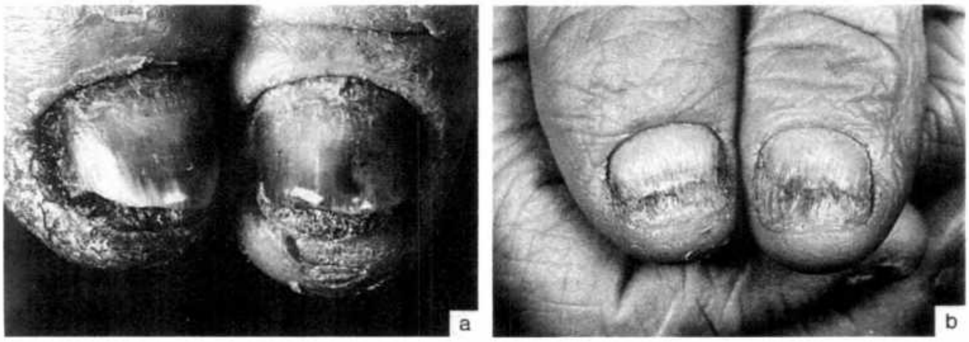

FIGURE 3. Case 1: (a) Appearance of nails before treatment. (b) Partial nail re-growth after 9 months of TP-5 treatment.

Case 2

A 69-year-old male, had had Sézary syndrome since January 1985, with exfoliative erythroderma, oedema of the lower limbs, palmoplantar keratoderma, nail dystrophy with subungual hyperkeratosis, generalized lymphadenopathy, splenomegaly, chemosis, severe itching and generalized herpes infection. At diagnosis the WCC was $9{\cdot}6 \times 10^{9}$/l with 34% neutrophils, 4% eosinophils, 8% lymphocytes, 4% monocytes and 50% SC ($4{\cdot}8 \times 10^{9}$/l). Progression of the clinical picture was observed in December of 1985, despite treatment with chlorambucil and prednisone. The WCC was $5{\cdot}9 \times 10^{9}$/l with 59% neutrophils, 10% eosinophils, 4% monocytes, 6% lymphocytes and 21% SC ($1{\cdot}2 \times 10^{9}$/l).

TP-5 was begun in December 1985. Itching disappeared and palmoplantar keratoderma regressed after one months therapy. A progressive clearing of the erythroderma was observed during the 2nd month. TP-5 therapy was then reduced to twice-weekly for a further 15 months. Six months after the commencement of TP-5, a skin biopsy showed a loss of epidermotropism and a reduction in the cell infiltrate compared with a pre-treatment biopsy. SC numbers in the peripheral blood remained unchanged until April 1986 ranging from 1·0 to $1{\cdot}5 \times 10^{9}$/l; their numbers then increased progressively. In September 1986 the WCC was $13{\cdot}5 \times 10^{9}$/l with 17% neutrophils, 3% lymphocytes and 80% SC ($10{\cdot}8 \times 10^{9}$/l). Despite these high SC numbers, a partial remission in the skin condition was maintained as confirmed by a further biopsy. A progressive decrease in WCC from 18·2 to $9{\cdot}1 \times 10^{9}$/l and in SC from 80% to 39% ($3{\cdot}5 \times 10^{9}$/l) with an increase in neutrophils from 17% to 49% was observed during the period September 1986–May 1987.

Case 3

A 72-year-old male had had Sézary syndrome since the spring of 1984, with erythemato-papular patches on the trunk and limbs and intense itching. In January 1985, erythroderma with generalized lymphadenopathy and hyperkeratosis of the palms and soles were observed, followed, 1 month later, by generalized herpes simplex infection. No significant improvement was obtained with mechlorethamine i.v., chlorambucil plus prednisone, Re-PUVA and VP-16-213. The WCC was $16{\cdot}6 \times 19^{9}$/l with 27% neutrophils, 33% eosinophils, 20% lymphocytes, 5% monocytes and 15% SC ($2{\cdot}5 \times 10^{9}$/l). All laboratory tests were within the normal range, apart from elevated LDH (585 IU).

TP-5 treatment was begun in November 1985. Reduction of oedema, clearing of erythroderma and complete remission of palmoplantar keratoderma became evident after 2 months therapy. TP-5 therapy was then continued twice weekly for 5 months. Itching was greatly reduced, LDH normalized and eosinophils were reduced from 33% to 17%. The treatment was then suspended for 1 month as both the clinical and histological picture showed an appreciable improvement, but had to be restarted at the initial dosage, due to the return of intense itching and increased skin thickening, with 43% eosinophils. At the time of writing the treatment had been continued for a further 11 months. During this period the WCC ranged from 10 to 13×10^{9}/l, total lymphoid cells decreased to $1{\cdot}2$–$1{\cdot}6 \times 10^{9}$/l and only a few Sézary cells were found in peripheral blood smears.

Case 4

The patient, a 73-year-old female, was hospitalized in November of 1986, with a 1-year history of erythroderma, generalized lymphadenopathy and intense itching. Since the onset of the disease the patient had been given only topical ointments. The WCC was $11{\cdot}4 \times 10^{9}$/l with 23% neutrophils, 2% basophils, 8% eosinophils, 4% monocytes, 2% lymphocytes and 61% SC

($7{\cdot}0 \times 10^9$/l). All laboratory tests were within the normal range. Skin histology confirmed the diagnosis of Sézary syndrome. TP-5 was administered at full dosage for 8 months. A progressive reduction of thickening and scaling was observed and this was confirmed histologically by a skin biopsy performed after 7 months. A progressive reduction in the WCC and SC numbers was also observed; after 6 months the WCC was $6{\cdot}4 \times 10^9$/l with 53% neutrophils, 2% basophils, 1% eosinophils, 9% monocytes, 16% SC ($1{\cdot}0 \times 10^9$/l) and 19% lymphocytes, many of which had abundant azurophilic granules and regular round nuclei. This haematological picture remained at the time of writing.

Side-effects

The only side-effects observed were two episodes of vasovagal syndrome (tachycardia, hypotension and an erythematous flush) during infusion in Cases 1 and 3. These did not require treatment nor alteration of the dosage. All patients sweated heavily during the first 10–15 days of treatment. No recurrences of herpes infection occurred.

METHODS

Peripheral blood studies

Differential counts and peripheral blood cell morphology were always evaluated by the same person (M.G.B.) on at least 300 cells. Cytospins prepared from Ficoll-Hypaque isolated mononuclear cells and stained with May-Grunewald-Giemsa were also examined in all cases, in order to obtain a comparison with peripheral blood smears. In addition, alpha-naphthyl acetate acid esterase, acid phosphatase and PAS stains were also used. Ficoll-Hypaque isolated mononuclear cells were pelleted and processed for electron microscopy.

The identification of Sézary cells (SC) was made on the basis of three characteristics: absence of cytoplasmic granules, nuclear density similar to or greater than that of normal lymphocytes, and the presence of nuclear grooves, splits convolutions or folds.

We chose for TP-5 treatment, only patients in whom SC represented at least 75% of all lymphoid cells, in order to work with the purest possible SC population.

Isolation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were isolated on a Ficoll-Hypaque density gradient. After washing three times with Hank's balanced salt solution (HBSS) the cells were resuspended at a density of 5×10^6 cells/ml in RPMI 1640 (Gibco, Grand Island Co., NY U.S.A.) supplemented with 5% heat-inactivated fetal calf serum (FCS). Cell viability was determined by the trypan blue exclusion test.

Monoclonal antibodies and analysis of lymphocyte subsets

The monoclonal antibodies used are listed in Table 1.

Lymphocyte subsets were counted using a standard immunofluorescence technique and an FACS 420 flow cytometer (Becton Dickinson Inc., Sunnyvale CA, U.S.A.).

Cell cultures

PBMC from the patients and 13 healthy controls were cultured as previously described,[10] and 200 μl volumes of cultures were incubated in triplicate with 0·2 μg phytohaemagglutinin (PHA), 2 μg pokeweed mitogen (PWM), 2 μg concanavalin A (ConA) or 50 U/ml recombinant interleukin-2 (IL-2) for 48 h and 72 h. Controls contained medium only.

TABLE 1. Monoclonal antibodies used in the study

Monoclonal antibody		Source*	Dilution	Reactivity‡
CD1†	OKT6	Ortho	1:25	Immature T cells, Langerhans cells
CD2	OKT11	Ortho	1:20	All E-rosette forming cells
CD3	OKT3	Ortho	1:20	Mature T cells
CD3	Leu-4	Becton Dickinson	1:50	Mature T cells
CD4	OKT4	Ortho	1:30	T helper/inducer subset
CD4	Leu 3	Becton Dickinson	1:15	T helper/inducer subset
CD5	Leu-1	Becton Dickinson	1:50	Pan T cells, B-CLL cells
CD7	Leu-9	Becton Dickinson	1:20	Pan T cells
CD8	OKT8	Ortho	1:40	T suppressor/cytotoxic subset
CD8	Leu-2a	Becton Dickinson	1:100	T suppressor/cytotoxic subset
nc**	OKT9	Ortho	1:20	Transferrin receptor; activated and proliferating cells
CD11	OKM1	Ortho	1:50	Monocytes, null cells, granulocytes
CD11	Leu-15	Becton Dickinson	1:50	Monocytes, null cells, granulocytes
nc	Leu-7 (HNK-1)	Becton Dickinson	1:30	Natural killer cells, some T cells
CD16	Leu-11	Becton Dickinson	1:20	Natural killer cells, granulocytes
nc	Leu-10	Becton Dickinson	1:50	HLA-DQ; B cells, monocytes
CD19	Leu-12	Becton Dickinson	1:20	Pan B cells
CD21	OKB7	Ortho	1:20	C3d receptor; B cells
CD22	Leu-14	Becton Dickinson	1:20	Pan B cells
nc	OKIa1	Ortho	1:200	HLA-DR; B cells, activated T cells
CD25	OKT26a	Ortho	1:20	IL-2 receptor; activated T cells and some B cells
CD28	Leu-8	Becton Dickinson	1:20	T cell regulatory subset
CD30	Ki-1	Dako	1:30	Activated T cells
nc	Ki-67	Dako	1:30	Activated T cells and proliferating cells
CD38	OKT10	Ortho	1:20	Precursor and activated T cells, immature T cells, plasma cells

*Ortho Pharmaceutical Corp., Raritan, NJ, U.S.A., Becton Dickinson Inc., Sunnyvale, CA, U.S.A., Dakopatts a/s, Copenhagen, Denmark.

†CD = cluster designation.

**nc = not clustered.

‡From Third International Workshop on Leukocyte Differentiation Antigens.[9]

Interferon-γ production

PBMC were cultured at a concentration of 2×10^5 cells in flat-bottomed microplate wells in 0·2 ml RPMI 1640 medium supplemented with 5% FCS and 1% PHA; or 1% PHA and 10 M 12-o-tetradecanoylphorbol-13-acetate (TPA) (Sigma). Control cells were incubated in RPMI 1640 with 5% FCS only. The microplates were incubated in a humidified 5% CO_2 atmosphere at 37°C for 36 h and then stored at −20°C. Levels of interferon-γ (IFN-γ) in the culture supernatants were assayed by measuring the protection of human amnion cells (WISH) from infection by vesicular stomatitis virus (VSV). A cytopathic effect assay in 96-well tissue culture microtitre plates was used, as previously described.[11]

Identification of the phenotype of skin infiltrates

Biopsies were taken from affected skin in all cases. The samples were divided, and one half was processed for routine paraffin histology and for electron microscopy. The other half was immediately embedded in compound OCT (Miles Laboratory, Naperville, IL, U.S.A.), snap frozen in liquid nitrogen at −150°C, and stored at −80°C. Cryostat sections (4–6 μm) were cut, air dried, and fixed in acetone for 10 min. Sections were stained using the avidin-biotin-peroxidase (ABC) technique[6,7] employing an ABC Vectastain Kit (Vector Laboratories Inc., Burlingame, CA, U.S.A.). Sections were also stained using the alkaline phosphatase technique.[8]

RESULTS

Histological findings

Prior to treatment, a typical band-like infiltrate in the upper dermis with strong epidermotropism of individual cells and Pautrier's abscesses were found in all cases. The infiltrate was composed of small and large hyperchromic cerebriform cells, identified as Sézary cells by electron microscopy. In Cases 1 and 3, lymph node involvement was confirmed histologically.

Two skin biopsies were taken from adjacent sites in all cases before and 6–7 months after the commencement of TP-5 treatment. A loss of epidermotropism and a reduction in cell infiltrate were observed (Fig. 4).

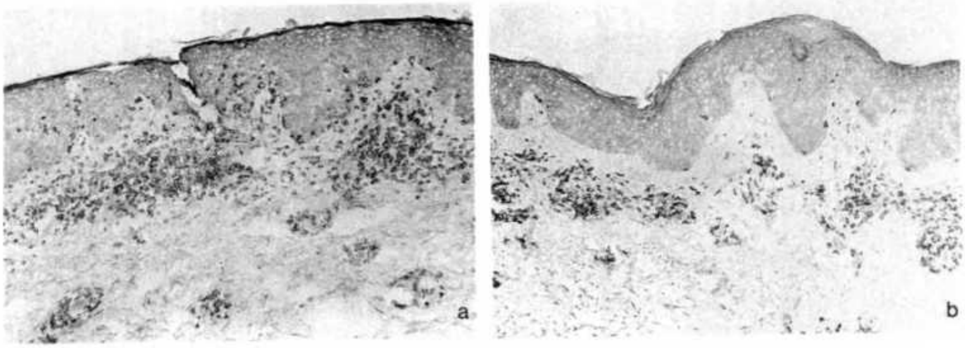

FIGURE 4. Case 2: Skin biopsy (a) before treatment and (b) after 6 months of TP-5 therapy. Note the loss of epidermotropism (Leu-3 antibody).

Immunological findings

Peripheral blood phenotype. The PBMC phenotypes at the onset of the disease and immediately before and after TP-5 treatment are listed in Table 2. In all patients, the T cell profile was examined every 2 months from diagnosis. During TP-5 therapy, this was examined on alternate days during the first 2 weeks and every 7–15 days thereafter. A progressive decrease in percentages and absolute numbers of CD4+ cells (CD4:CD8+ ratio < 7) was noted after 2 months in Cases 1, 3 and 4. These changes were maintained for 10, 12 and 8 months, respectively. An increase in the percentages and absolute numbers of CD8+ cells was observed in Cases 1 and 2. An increase in interleukin-2 (IL-2) receptors on PBMC was seen between the first and 2nd month of treatment from 4% ($0{\cdot}4 \times 10^9$/l; Case 1), 3% ($0{\cdot}05 \times 10^9$/l; Case 2) and 3% ($0{\cdot}1 \times 10^9$/l; Case 3) to 55% ($0{\cdot}6 \times 10^9$/l), 42% ($0{\cdot}7 \times 10^9$/l) and 35% ($0{\cdot}6 \times 10^9$/l) respectively. However, a reduction to around 20% was observed in the 4th month. In Case 4 no increase in CD25+ cells was observed. An increase in HNK-1 (Leu-7) positive cells was also observed after 2 months in Cases 1 and 2 and this was maintained for about 6 months. CD16+ cells increased in Cases 2 and 3.

Proliferative response. A poor response to all mitogens was observed when Sézary cells were

TABLE 2. Phenotypes of PBMC, at the time of diagnosis, and immediately before and after TP-5 treatment in four patients with Sézary syndrome

		Cell counts × 10⁹/l			% positive cells											
Case no.	Time of examination	Total white cells	Lymphoid cells	Sézary cells	CD3	CD4	CD7	CD8	CD16	H-NK1	CD25	CD28	T9	CD38	HLA-DR	anti-B
1	At diagnosis	13·0	3·4	3·3	99	98	58	1	1	1	ND	88	1	2	2	2
	Before TP-5	21·2	8·1	8·0	99	98	44	1	< 1	< 1	4	88	1	3	< 1	1
	After 6 months	6·9	1·0	0·1	66	45	50	24	5	26	17	43	4	3	18	9
2	At diagnosis	9·6	5·6	4·8	89	67	50	6	10	5	2	40	< 1	< 1	6	9
	Before TP-5	5·9	1·6	1·2	89	63	62	17	10	5	3	52	5	5	12	9
	After 1 month	6·3	1·8	1·1	90	68	59	26	10	23	20	56	3	11	8	8
	After 7 months	13·4	11·2	10·8	89	88	11	4	3	3	3	64	< 1	< 1	2	
3	At diagnosis	13·4	3·5	2·4	85	73	49	16	2	15	3	60	2	4	6	2
	Before TP-5	16·6	3·3	2·5	81	57	64	21	2	16	3	60	2	6	12	6
	After 7 months	8·5	1·6	0·1	73	47	63	26	15	33	20	67	2	< 1	15	11
4	Before TP-5*	11·4	7·2	6·2	96	85	76	10	10	12	5	80	1	3	< 1	5
	After 6 months	6·4	2·2	1·0	93	80	81	11	ND	6	3	71	< 1	< 1	12	3

*Case 4 was treated immediately after diagnosis

TABLE 3. PBMC proliferative response to mitogens and IL-2, before and after TP-5 treatment in four patients with Sézary syndrome. Values are counts/min (mean ± SD for triplicate determinations)

Case no.	Time of testing	Medium alone	PHA	PHA + IL-2	IL-2	ConA	Pokeweed mitogen
1	Before treatment	417 ± 75	376 ± 15	10212 ± 377	670 ± 50	319 ± 63	263 ± 29
	After 3 months	130 ± 46	16226 ± 1894	31232 ± 308	3297 ± 1075	ND	ND
	After 7 months	165 ± 50	32853 ± 1150	30831 ± 2100	1583 ± 850	17775 ± 853	1731 ± 225
2	Before treatment	107 ± 10	370 ± 47	12910 ± 1176	596 ± 43	240 ± 43	84 ± 5
	After 7 months	101 ± 16	16458 ± 1524	33781 ± 2610	1583 ± 250	880 ± 82	1296 ± 95
3	Before treatment	310 ± 58	818 ± 28	ND	1005 ± 259	574 ± 122	6830 ± 239
	After 1 month	149 ± 6	3177 ± 253	35795 ± 4713	3057 ± 82	ND	ND
	After 6 months	411 ± 150	6890 ± 450	10040 ± 596	1133 ± 348	2196 ± 450	4042 ± 850
4	Before treatment	84 ± 23	1851 ± 391	15122 ± 545	770 ± 134	1048 ± 121	803 ± 33
	After 6 months	331 ± 53	4270 ± 388	10551 ± 436	1966 ± 97	ND	ND
Normal controls ($n = 13$)		236 ± 529	22830 ± 6066	23830 ± 7066	4386 ± 1674	22513 ± 3980	26213 ± 13373

ND = not determined

tested at the onset of the disease and before TP-5 therapy (Table 3). The addition of recombinant IL-2 to PHA led to an increase in the proliferative response. SC from all cases did not respond directly to IL-2 and, like normal T lymphocytes, they did have an absolute requirement for prior lectin activation. During treatment, in all cases, lymphoid cells showed an increase in the proliferative response to mitogens, beginning after 3 months (Table 3). This normalization in the proliferative response in Cases 1 and 3 could be attributed to the presence of normal cells. In Case 2, however, where SC represented 80% of the white blood cells and 96% of the lymphoid cells, phenotypic FACS analysis of 48 h PHA-stimulated cultured cells showed 90% CD4+, 11% CD8+, 56% HLA-DR+, 77% CD25+ cells. Furthermore, cytospins from these cultures showed blasts with SC morphology and CD4+ phenotype.

In vitro effect of TP-5. We tested the *in vitro* effect of TP-5 alone and TP-5 plus PHA at different TP-5 concentrations (1, 100, 1000 μg/ml) on PBMC from Cases 1, 3 and 4 after 1, 3 and 6 months, therapy, respectively. The same tests were carried out on PBMC from an untreated case of Sézary syndrome who was in a leukaemic state (white cell count $30{\cdot}3 \times 10^9$/l with $22{\cdot}1 \times 10^9$/l SC) and from seven healthy controls.

TP-5 alone showed no effects at any concentration, while the combination of TP-5 (at the optimal dose of 10 μg/ml) and PHA induced an increment in the proliferative response only in the Sézary syndrome patients (Table 4).

TABLE 4. *In vitro* PBMC proliferative response to TP-5, PHA and PHA plus TP-5 in four patients with Sézary syndrome and seven healthy controls. Values are counts/min (mean ± SD of triplicate determinations)

Case no.	Medium alone	TP-5	PHA	PHA + TP-5
1	170 ± 16	337 ± 67	6277 ± 440	10896 ± 626
3	162 ± 41	270 ± 94	2957 ± 150	4640 ± 422
4	331 ± 53	163 ± 78	4270 ± 388	5127 ± 506
Sézary patient before chemotherapy	65 ± 9	76 ± 15	2792 ± 360	6770 ± 387
Normal controls ($n=7$)	243 ± 127	247 ± 100	21721 ± 5192	21021 ± 3731

Activation antigens. Before therapy, the activation antigens OKT9, CD38, HLA-DR and CD25 were virtually absent in resting cells from all four cases. PHA stimulation induced only the expression of IL-2 receptors in 23·7 ± 14·4% of cells. After treatment, PHA-stimulated PBMC were positive for all these antigens with values comparable to those observed in normal controls (Table 5).

IFN-γ production. Interferon-gamma (IFN-γ) production by PBMC from our four cases is shown in Table 6. Before treatment, IFN-γ production by lymphocytes stimulated with PHA or PHA plus TPA, was markedly lower than in normal controls. IFN-γ production increased to normal values after therapy.

Skin immunohistochemistry. Before treatment, the phenotype of the lymphoid cells in the

TABLE 5. Expression of activation antigens on 48 h PHA stimulated PBMC from four patients with Sézary syndrome, after TP-5 treatment

		% positive cells			
Case no.		OKT9	CD38	CD25	HLA-DR
1	Medium alone	7	1	1	10
	PHA	40	71	82	80
2	Medium alone	1	1	3	3
	PHA	10	8	77	56
3	Medium alone	5	3	18	10
	PHA	8	44	59	35
4	Medium alone	7	6	5	5
	PHA	36	16	29	6
Normal controls	Medium alone	4 ± 3*	12 ± 8	5 ± 1	11 ± 7
(*n* = 9)	PHA	25 ± 19	55 ± 12	57 ± 18	38 ± 11

*Mean ± SD

TABLE 6. Production of IFN-γ by PBMC from four patients with Sézary syndrome, before and after TP-5 treatment

		IFN-γ IU/ml		
Case no.	Time of testing	Control	PHA	PHA + TPA
1	Before treatment	0	4	8
	After 7 months	32	64	256
2	Before treatment	0	4	8
	After 7 months	4	64	> 256
3	Before treatment	4	16	64
	After 6 months	4	64	> 256
4	Before treatment	0	4	64
	After 6 months	0	64	> 256
Normal controls (*n* = 9)		0–8	32–128	128–512

dermal infiltrate and in the epidermis was always similar to that in the peripheral blood: CD3+, CD4+, CD5+, CD8−. After treatment, CD4+ cells disappeared from the epidermis in all cases (Fig. 4) and CD8+ cells increased in Cases 1, 2 and 3. The most notable finding was the dramatic reduction of Langerhans cells in the epidermis and dermis (Fig. 5) and the reduction of HLA-DR expression on Langerhans cells and keratinocytes. In all cases, there were 20–30% skin CD25+ cells and 1–2% HNK-1+ dermal lymphoid cells before therapy. In Cases 1, 2 and 3 the former decreased to 2–5% and the latter accounted for about 10% after 6–7 months of therapy.

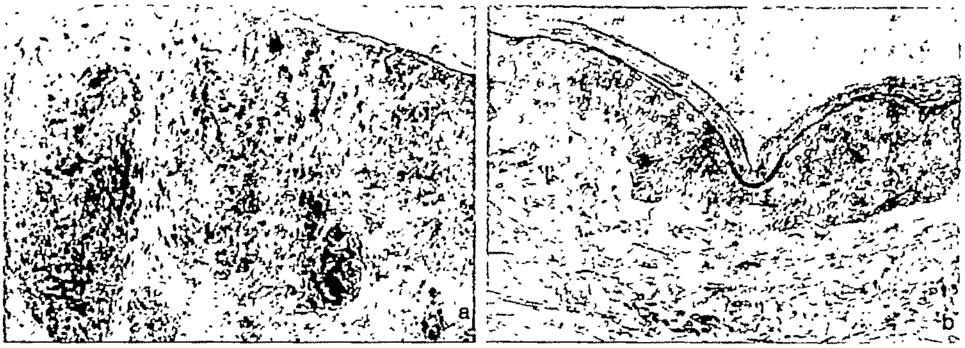

FIGURE 5. Case 3: CD1+ Langerhans cells in skin biopsies taken (a) before treatment and (b) after 6 months TP-5 therapy. (OKT6 antibody).

DISCUSSION

It has been demonstrated that thymopoietin and the synthetic pentapeptide thymopentin (TP-5)[12] have numerous immunological activities including promotion of T-cell differentiation,[13] regulation of several immune responses,[14,15] and an influence on granulocyte differentiation.[16] Our preliminary results indicate that TP-5 is also clinically, histologically, and immunologically active in Sézary syndrome.

Objective clinical improvement was noted in our four cases. The response was dramatic in Case 1. The reduction in skin thickening corresponded histologically to a reduced infiltrate and loss of epidermotropism. The most striking histological change was the net reduction in the number of Langerhans cells, both in the epidermis and dermis, showing that TP-5 is directly or indirectly active against one of the elements most frequently implicated in the pathogenesis of cutaneous T-cell lymphomas.[17]

Przybilla *et al.*[18] treated one patient with mycosis fungoides with TP-5 i.v. at the dose we used in our study and seven patients (five with mycosis fungoides, one with Sézary syndrome and one with low-grade malignant lymphoma) with TP-5 subcutaneously. These authors found a moderate improvement in three of eight patients whilst three worsened. We used i.v. administration in order to increase the activity of suppressor or cytotoxic cells or both against the proliferation of CD4+ atypical cells.

As the Sézary cells decreased, the immunological profiles changed. The most intriguing effects were the increase in functional activities (response to mitogens and interferon-γ production), and the acquisition of membrane activation antigens. In two cases, these modifications may have been due to the activity of normal cells (CD4+ or CD8+ lymphocytes) present in larger numbers after treatment. However, in Case 2 the Sézary cells represented 80% of white cells and 96% of lymphoid cells and these modifications must have depended, at least partially, on some functional capacity acquired by the atypical cells. When neoplastic cells are present in large quantities, the normal counterpart may not be able to kill or inhibit the proliferation of neoplastic clones. However the presence of antimitotic drugs may improve this. Accumulating evidence indicates that proliferation of T cells is regulated by interleukin-2 (IL-2) and its membrane receptor.[19–22] The IL-2 receptor, recognized by the anti-CD25 monoclonal antibody, is expressed on T cells activated by lectins or antigens, but not on resting T cells.[23] Our observations showed that the IL-2 receptor was induced on the PBMC of Sézary syndrome patients after 2 months TP-5 treatment. The increase in IL-2 receptors and acquisition of proliferative capacity may indicate that the cells increased their IL-2 production. Solbach *et al.*[24] and our previous results[11,25] have shown that Sézary cells are not able to produce IL-2 spontaneously. We have also observed that exogenous IL-2 was able to increase IFN-γ production by Sézary cells.[11,25] This suggests that these cells suffer from an intrinsic defect that can be partly corrected by the addition of recombinant IL-2. In all cases, addition of IL-2 to the culture medium containing PHA led to a higher proliferative response. We conclude that SC are not able to produce IL-2, whilst they can respond to it. Furthermore, SC do not generally respond directly to IL-2 and, like normal T lymphocytes, they have an absolute requirement for prior lectin or antigen activation. In this respect SC behave like PBMC from immunodeficient patients. However, we have observed a case with an aggressive course and an unusual SC phenotype (CD4+, HNK-1+), where the stimulation of SC with IL-2 was followed by a proliferative response even without mitogens.[11] The *in vitro* response to IL-2 alone could thus be useful to differentiate subgroups of Sézary syndrome with a different clinical course.

In *in vitro* culture, Sézary syndrome PBMC did not proliferate upon TP-5 stimulation, however, simultaneous stimulation with PHA and TP-5 led to a higher proliferative response than with PHA alone. TP-5 did not alter the proliferative activity of PBMC from normal controls. Rubenfeld *et al.*[26] reported the induction of lymphocyte differentiation by epidermal cultures. Chu *et al.*[27] demonstrated a thymopoietin-like substance in normal human epidermal cells. Our results suggest that, in the skin, SC under the influence of epidermal thymus-like factors can increase their IL-2 production or take up IL-2 produced by activated normal T cells and utilize it to proliferate.

Tac-positive (i.e. CD25+) cells have been demonstrated in the circulation after immunization *in vivo* with tetanus toxins; however, this represents a very transient appearance of such cells.[28] Lotze *et al.*[29] showed that fresh peripheral blood lymphocytes incubated *in vitro* in medium containing IL-2 kill fresh, noncultured, human tumour cells. Ito *et al.*[30] demonstrated that pre-incubation of peripheral blood non-adherent mononuclear cells with purified or recombinant IL-2 significantly enhanced NK activity against varicella zoster virus-infected targets. Allavena *et al.*[31] showed that exposure of a culture of lymphocytes from T lymphoproliferative disorders to IL-2 for 3–5 days resulted in high levels of cytotoxic activity. In addition, IL-2 can induce IFN-γ secretion[32–34] and it has been suggested that it is responsible for augmentation of NK activity.[35,36] On the other hand, recent findings indicate that stimulation of human natural cytotoxicity by IL-2 is at least to some extent independent of induction of IFN-γ secretion.[37]

TP-5 has been demonstrated to enhance murine splenic cytotoxic precursor units after *in vitro* and *in vivo* suboptimal antigenic stimulation.[15] The results of Fiorilli *et al.*[38] suggest that TP-5 may enhance bone marrow NK cells by allowing the maturation of their precursors. The synthesis of other cytotoxic factors, such as tumour necrosis factors (TNF) α and β, are induced by IL-2 and IFN-γ. Interferon-γ, in combination with IL-2, resulted in a fivefold increase in TNF-α and -β.[39]

In our series, HNK-1+ cells increased after the transient increase in IL-2 receptors and at the same time as the increase in IFN-γ production *in vitro* and the clinical remission. This suggests that clinical response in TP-5 treated patients may be mediated by an increased production of IL-2 and consequent generation of cytotoxic cells and possibly lymphokines able to increase NK activity. In a randomized open controlled trial using TP-5 in stage I cutaneous melanoma,[40] we found a significant percentage and absolute increase in numbers of lymphocytes bearing IL-2 receptors only in the TP-5 group after 2 months treatment. In TP-5 treated patients a significant increase in HNK-1+ and CD16+ cells was also observed after 2 months.

Our results suggest that TP-5 can regulate immune responses by acting on regulatory subpopulations or on control circuits of cell-to-cell communication.

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Improvement of psoriasis by a topical vitamin D_3 analogue (MC 903) in a double-blind study

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The effect of the non-calciotropic vitamin D_3 analogue MC 903 on psoriasis vulgaris was assessed in a double-blind, placebo controlled trial in 30 patients. Lesions on one side of the body were treated for 6 weeks with a cream containing 10 μg/g, 33 μg/g or 100 μg/g MC 903 and lesions on the other side were treated with the cream base alone, according to a randomized design. Nine of the 10 patients in each treatment group completed the study. MC 903 cream gave a statistically significant decrease in erythema, thickness and scaling of the lesions, compared with the control. Overall assessment of psoriasis after 6 weeks showed moderate or excellent improvement in two of nine patients treated with 10 μg/g, in five of nine patients treated with 33 μg/g, and in seven of nine patients treated with 100 μg/g MC 903. Placebo treatment showed a moderate improvement in only one of the 27 patients. The histopathological picture of the psoriatic lesions corresponded with the clinical changes. The patients reported no adverse reactions, and laboratory tests did not show any significant changes; in particular there was no change in serum calcium levels. These results suggest that the vitamin D_3 analogue MC 903 is an effective and safe topical treatment for psoriasis.

Although much emphasis has been placed on the inflammatory and immunological changes occurring in psoriatic skin, epidermal hyperproliferation and incomplete epidermal differentiation remain the best markers of psoriasis. Attempts to normalize epidermal growth may, therefore, result in an improvement in the disease. A growing body of evidence indicates that 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2$-D_3), the naturally occurring, active form of vitamin D_3, can control the proliferation and differentiation of several cell types, including epidermal keratinocytes.[1,2] In cultures of human keratinocytes 1α,25-$(OH)_2$-D_3 causes a dose-dependent decrease in proliferation and an increase in morphological differentiation.[3]

It has already been reported that psoriasis improves after topical administration of 1α,24-$(OH)_2D_3$[4] and 1α,25-$(OH)_2$-D_3[5] and after oral administration of 1α,-OH-D_3.[5] However, these clinical trials were open and without controls. It is also a matter of concern that 1α,25-$(OH)_2$-D_3

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is a calcium regulating hormone.[6] Consequently, administration of $1\alpha,25\text{-(OH)}_2\text{-D}_3$ may result in the development of hypercalcaemia and hypercalcuria. Approximately 50% of preleukaemic patients treated with $1\alpha,25\text{-(OH)}_2\text{-D}_3$ orally developed hypercalcaemia.[7]

MC 903 is a novel vitamin D analogue which is as potent as $1\alpha,25\text{-(OH)}_2\text{-D}_3$ in inducing cell differentiation and in inhibiting cell proliferation, but at least 100 times less active than $1\alpha,25\text{-(OH)}_2\text{-D}_3$ in its effects on calcium metabolism.[8] The aim of the present study was to determine the effect of topical application of MC 903 on psoriasis in a double-blind, placebo controlled trial.

METHODS

Patients

Thirty out-patients with moderate, chronic plaque-type psoriasis participated in the study; patients with other types of psoriasis were excluded. Patients were at least 18 years of age, had symmetrically distributed psoriatic plaques which had been stable in extent and severity for at least 2 weeks, and had not used systemic, intralesional or ultraviolet irradiation therapy for at least 2 months or topical therapy except bland emollients for at least 2 weeks before the study. Women participated only if they were above childbearing age or were using adequate contraception. All patients had normal serum levels of calcium and creatinine. No patient was taking calcium tablets. Patients who were already taking one multivitamin tablet daily containing 200–450 IU vitamin D (the recommended daily dose) were allowed to continue to do so.

Study design

We conducted a randomized, double-blind study to compare three different concentrations of MC 903 (Leo Pharmaceutical Products, Copenhagen, Denmark) with placebo. MC 903 was formulated at concentrations of 10 μg, 33 μg and 100 μg/g vehicle consisting of a water-in-oil emulsion. The patients were treated twice daily with MC 903 cream applied to the plaques on one side of the body and vehicle only to those on the other side according to a randomized design. Each patient was treated with only one concentration of the MC 903 cream.

Clinical evaluation of the psoriasis was made by the same physician at weeks 0,1,2,4 and 6. Plaques were graded for erythema, scaling and thickness as follows: absent, 0; minimal, 1; moderate, 2; or severe, 3. This grading was done separately for upper extremities, trunk and lower extremities. For each criterion the mean score for the three body areas was used for statistical analysis. To evaluate the overall response of the psoriasis to therapy, the physician also determined a global score at each visit, based on an overall assessment of the patient's psoriasis including extent as well as severity in comparison with the clinical condition at week 0: deterioration, −1; no change, 0: slight improvement, 1; moderate improvement, 2; and excellent improvement, 3. The patient was asked to assess the overall clinical response in the same way.

At each visit a blood sample was taken for a full blood count, and measurement of serum alkaline phosphatase, aspartate amino-transferase, creatinine, ionized calcium and phosphate, and a urine sample for measurement of albumin and glucose. At weeks 0, 1 and 6, a 4 mm skin biopsy was taken from one lesion on each side of each patient under local anaesthesia with 1% lidocaine containing 1:1000 adrenalin. The specimens were stained with haematoxylin and eosin and graded for acanthosis, parakeratosis, suprapapillary thinning, neutrophil exocytosis, papillary vessel dilatation and tortuosity and dermal mononuclear leukocyte infiltration on a scale from 0 to 3 where 0 indicated absent and 3 indicated severe.

TABLE 1. Details of the 30 psoriatic patients included in the study. Values are means and ranges

	MC 903		
	10 μg/g	33 μg/g	100 μg/g
Sex	6M 4F	10F	3M 7F
Age (years)	38 (23–64)	42 (18–65)	37 (20–54)
Duration of psoriasis (years)	14 (5–25)	17 (2–35)	15 (1–32)
Percentage body surface involved	17 (12–25)	17 (13–24)	22 (15–50)
Clinical scores			
Erythema	2·4 (1·3–3)	2·4 (1·5–3)	2·6 (1·7–3)
Thickness	2·1 (1·3–3)	2·1 (1·5–3)	2·5 (1·7–3)
Scaling	2·1 (1·3–3)	2·3 (1·5–3)	2·3 (1·3–3)

Statistical analysis

The assessment of statistical significance was based on non-parametric tests only.

In each treatment group the results with MC 903 and placebo were compared using a Wilcoxon matched pairs signed ranks test. The results for the three different concentrations of MC 903 were compared using a Kruskal-Wallis non-parametric one-way analysis of variance.

RESULTS

After breaking the code at the end of the trial, it was found that the three treatment groups were comparable with respect to age and duration and initial severity of psoriasis; however, the sex ratios were different as only female patients had been assigned to treatment with 33 μg/g MC 903 (Table 1). Twenty seven of the 30 patients completed the study. Three patients (one in each treatment group) defaulted. The scores for erythema, infiltration and scaling over 6 weeks in patients receiving 10 μg/g, 33 μg/g or 100 μg/g MC 903 are shown in Figures 1, 2 and 3, respectively. Only minor and not statistically significant improvement occurred in lesions

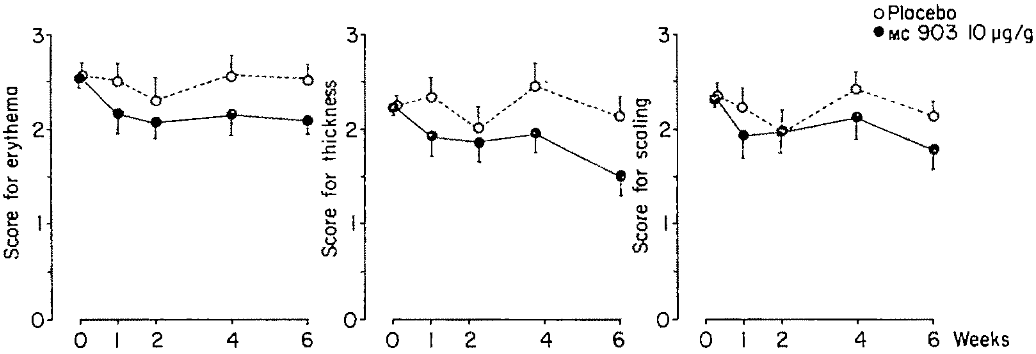

FIGURE 1. Changes in erythema, thickness and scaling of psoriatic lesions treated with MC 903 10 μg/g (——) and vehicle alone (- - -). Values are means ± SEM, ($n = 9$).

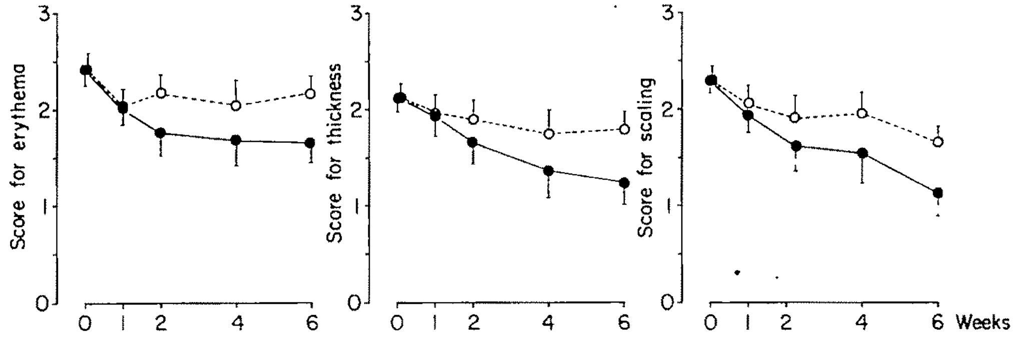

FIGURE 2. Changes in erythema, thickness and scaling of psoriatic lesions treated with MC 903 33 μg/g (——) and vehicle alone (- - -). Values are means ± SEM, ($n=9$).

treated with vehicle alone. Lesions treated with MC 903 showed a reduction in erythema, thickness and scaling, but complete clearance was not achieved in any of the patients. At the end of treatment, the clinical scores with 33 μg/g and 100 μg/g MC 903 were significantly better than with placebo ($P<0\cdot05$ for erythema, thickness and scaling). The lowest concentration of MC 903 (10 μg/g) did not produce significant improvement compared with vehicle alone ($P>0\cdot05$). The degree of improvement tended to be different in the three treatment groups. However, this difference was only statistically significant for scaling between 10 μg/g and 100 μg/g MC 903 ($P=0\cdot02$).

Most psoriatic lesions treated with 33 μg/g or 100 μg/g MC 903 showed moderate to excellent improvement. In patients showing excellent improvement a slight erythema persisted after treatment for 6 weeks. Figure 4 shows a patient whose improvement on the MC 903 treated side was classified as moderate.

The investigator's overall assessment of the clinical response to MC 903 and vehicle is shown in Figure 5. Only one of 27 patients showed a moderate improvement on the vehicle treated side. A moderate or excellent improvement was obtained in two of nine patients treated with 10 μg/g MC 903 ($P>0\cdot05$), in five of nine patients treated with 33 μg/g MC 903 ($P<0\cdot05$), and in seven of nine patients treated with 100 μg/g MC 903 ($P<0\cdot05$). The differences in the degree of improvement between the three treatment groups were not statistically significant.

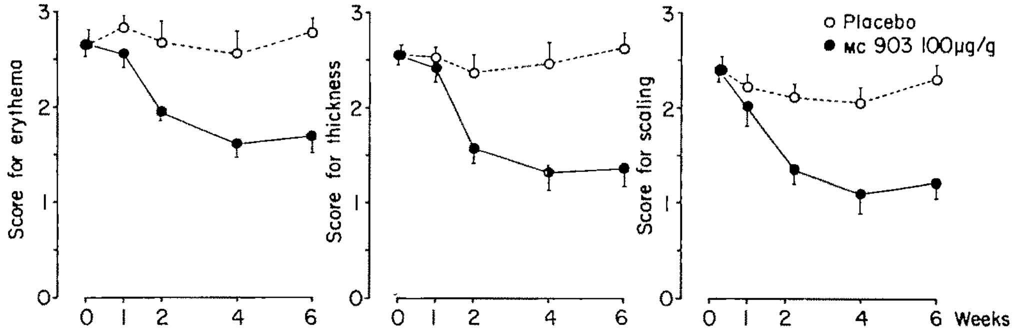

FIGURE 3. Changes in erythema, thickness and scaling of psoriatic lesions treated with MC 903 100 μg/g (——) and vehicle alone (- - -). Values are means ± SEM, ($n=9$).

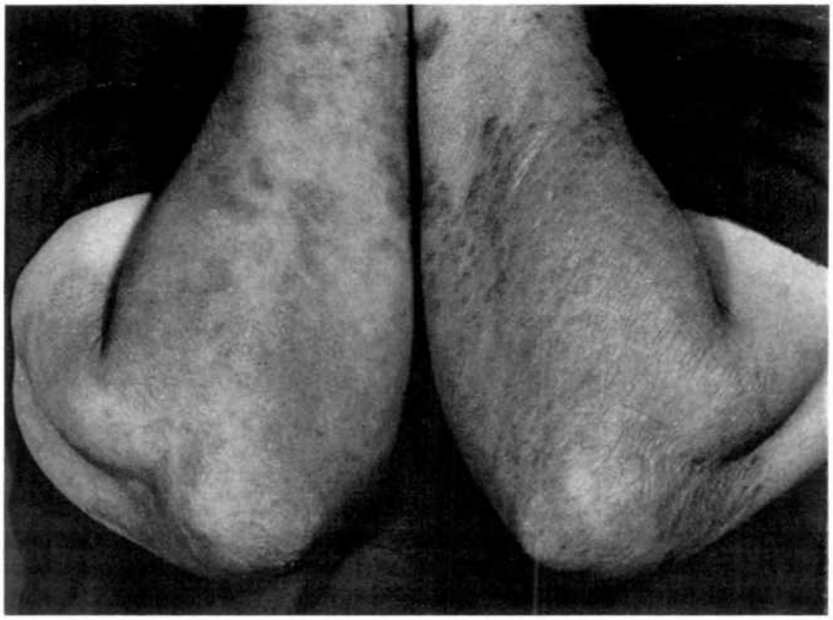

FIGURE 4. Psoriatic lesions treated with MC 903 100 μg/g (left) and vehicle alone (right) for 6 weeks.

Patients did not report any adverse reactions, either local or systemic. One patient treated with 33 μg/g MC 903 had increased serum levels of alkaline phosphatase and aspartate aminotransferase (each twice the upper limit of the normal range). However, these abnormalities were present before starting therapy, and no further increases were observed during therapy. All other laboratory tests were normal. In particular, no significant changes in serum ionized calcium were detected (Table 2); serum calcium levels did not increase above the upper limit of the normal range in any patient at any time point during the study.

The histological picture in the biopsies taken from the psoriatic lesions correlated with the clinical changes. In lesions which were improving during treatment with MC 903 there was a decrease in the degree of epidermal acanthosis, parakeratosis, suprapapillary thinning,

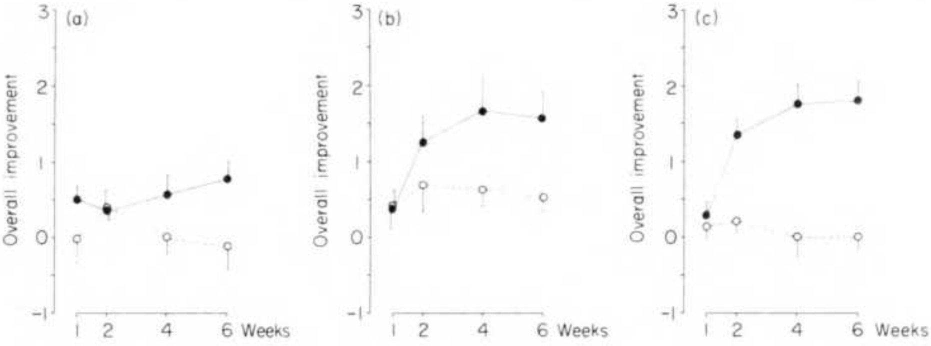

FIGURE 5. Investigator's assessment of the overall clinical response to MC 903 (——) and vehicle alone (- - -). (a) 10 μg/g MC 903, (b) 33 μg/g MC 903 and (c) 100 μg/g MC 903. Values are means ± SEM for nine patients in each treatment group.

TABLE 2. Serum ionized calcium levels during treatment with MC 903

	Serum ionized calcium (mmol/l) (mean ± SEM)		
Week	MC 903 10 μg/g ($n=9$)	MC 903 33 μg/g ($n=9$)	MC 903 100 μg/g ($n=9$)
0	1·24 ± 0·01	1·24 ± 0·01	1·23 ± 0·01
1	1·23 ± 0·01	1·23 ± 0·01	1·21 ± 0·02
2	1·24 ± 0·01	1·21 ± 0·01	1·22 ± 0·01
4	1·24 ± 0·02	1·23 ± 0·01	1·22 ± 0·01
6	1·24 ± 0·01	1·23 ± 0·01	1·22 ± 0·02

Normal range for serum ionized calcium: 1·15–1·30 mmol/l.

neutrophil exocytosis, dermal infiltration of mononuclear leukocytes and papillary vessel dilatation and tortuosity (Fig. 6). These changes were detectable after 1 week, but were more pronounced after 6 weeks. Furthermore, after 1 week there was a decrease in the severity scores for all the histopathological parameters; in particular there was no evidence that the abnormal epidermal growth pattern had started to normalize earlier than the inflammatory and vascular changes. In no patient did the histological picture show a complete return to normal. Even in those patients responding most favorably to MC 903 a mild acanthosis was still present.

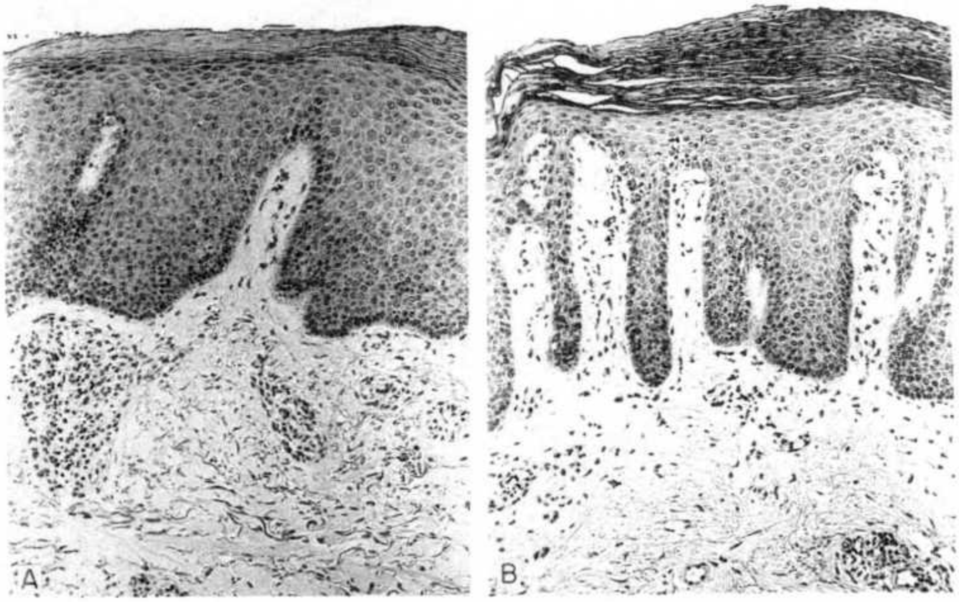

FIGURE 6. Punch biopsies from psoriatic lesions treated with (A) 100 μg/g MC 903 and (B) vehicle alone, for six weeks. (H & E; original × 250).

DISCUSSION

This is the first controlled study, to our knowledge, to show that a topically applied vitamin D_3 analogue improves psoriasis vulgaris. We used the vitamin D_3 analogue MC 903, which, in contrast to the naturally occurring $1\alpha,25\text{-(OH)}_2\text{-D}_3$, has minimal, if any, effect on the calcium balance.[8] The improvement obtained with MC 903 was detectable after 1 week both clinically and histopathologically and increased during the 6-week treatment period. The highest concentrations of MC 903 produced a moderate or excellent improvement in most patients without clinical or biochemical side-effects.

Previously, two open clinical studies conducted in Japan have suggested the possible value of vitamin D_3 analogues in psoriasis. Morimoto *et al.*[5] treated psoriatic patients with topical $1\alpha,25\text{-(OH)}_2\text{-D}_3$ under 12 h occlusion at a concentration of 0·5 μg/g vehicle. Out of their 19 patients, 16 improved within 3 weeks. Kato *et al.*[4] used a new synthetic analogue $1\alpha,24\text{-(OH)}_2\text{-D}_3$ reported to be as active as natural $1\alpha,25\text{-(OH)}_2\text{-D}_3$ in mineral metabolism.[9] The application of $1\alpha,24\text{-(OH)}_2\text{-D}_3$ at a concentration of 4 μg/g vehicle under 12 h occlusion resulted in clearance of 10 of 15 test sites within 1–4 weeks. Morimoto *et al.*[5] also treated some psoriatic patients with oral $1\alpha\text{-OH-D}_3$, which is metabolized to $1\alpha,25\text{-(OH)}_2\text{-D}_3$ in the liver.[10] At a dose of 1 μg/day, $1\alpha\text{-OH-D}_3$ was effective in 13 of 17 patients, although only after 3 months of therapy. Thus, it appears that active forms of vitamin D_3 are more effective topically than orally.

In psoriatic patients receiving oral $1\alpha\text{-OH-D}_3$ a significant increase of the serum calcium level has been found.[5] Although this increase was slight, these results may indicate that long-term treatment with vitamin D_3 analogues could be accompanied by hypercalcaemia and hypercalciuria. This serious side-effect is extremely unlikely to occur with MC 903. After oral and intraperitoneal administration to rats, MC 903 was found to be at least 100 times less active than $1\alpha\text{-OH-D}_3$ in promoting calcium excretion in the urine.[8] The same difference in potency was observed when MC 903 was compared with $1\alpha\text{-OH-D}_3$ with respect to production of changes in bone calcium metabolism and production of hypercalcaemia.[8] At the same time, MC 903 has been found to be almost as potent as $1\alpha,25\text{-(OH)}_2\text{-D}_3$ in inducing differentiation and inhibiting proliferation of a human histiocytic lymphoma cell line U937.[8] These biological activities make this compound an ideal candidate for studies on human proliferative skin disorders. The results of the present study have demonstrated that topical MC 903 may be an effective and safe treatment for psoriasis.

Although the rationale for using vitamin D_3 and its analogues in the treatment of psoriasis is to reverse the abnormal epidermal growth pattern, alternative mechanisms of action are possible. Recently $1\alpha,25\text{-(OH)}_2\text{-D}_3$ has been shown to possess immunoregulatory properties *in vitro*. Resting monocytes and activated B and T lymphocytes have receptors for $1\alpha,25\text{-(OH)}_2\text{-D}_3$[3] and picomolar concentrations of $1\alpha,25\text{-(OH)}_2\text{-D}_3$ inhibit the proliferation of T lymphocytes stimulated with antigens and mitogens.[12,13] Also, the release of interleukin 2 by mixed T lymphocytes activated by a mitogen is inhibited by $1\alpha,25\text{-(OH)}_2\text{-D}_3$.[14] Because a growing body of evidence supports the idea that immunological injury involving activated T helper cells may be an important mechanism in psoriasis,[15–18] it is conceivable that $1\alpha,25\text{-(OH)}_2\text{-D}_3$ exerts its anti-psoriatic effect in part by modulating T lymphocyte activity.

While there is no experimental evidence to support a direct anti-inflammatory effect of $1\alpha,25\text{-(OH)}_2\text{-D}_3$, studies with a murine T helper cell clone have shown that $1\alpha,25\text{-(OH)}_2\text{-D}_3$ inhibits the proliferative effect of interleukin 1.[19] This antagonistic effect of $1\alpha,25\text{-(OH)}_2\text{-D}_3$ on interleukin 1 driven activity is of potential importance, because psoriatic lesions contain biologically active amounts of interleukin 1-like material.[20] Whether $1\alpha,25\text{-(OH)}_2\text{-D}_3$ also inhibits the interleukin 1 induced stimulation of epidermal cell proliferation[21] and induction of skin inflammation[22] remains to be investigated.

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Cryotherapy for skin cancer: results over a 5-year period using liquid nitrogen spray cryosurgery

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SUMMARY

Three hundred and ninety five selected non-melanoma skin cancers were treated and followed up during the period 1981–1986, using a standardized cryosurgical technique. There was an overall cure rate of 97%. Recurrences were seen in 6 out of 225 (2·7%) treated basal cell carcinomas at a median time of 18 months, 1 of 34 (2·9%) squamous cell carcinomas, with a recurrence at 6 months post-operatively and 1 out of 128 (0·8%) of Bowen's tumours which recurred 6 months after treatment. The patients were treated as out-patients using local anaesthesia and all tolerated the treatment well.

This study has shown that cryosurgery is a safe, low cost and effective method of treating selected non-melanoma skin cancer and produced excellent cosmetic results with a high cure rate. It is suggested that patients treated with cryosurgery are followed up carefully for 2 years post-operatively.

Skin cancer is the most common malignancy in man and its early detection and effective treatment produces a high cure rate. There is now considerable evidence suggesting that the aetiology of basal cell carcinoma and squamous cell carcinoma is associated with long-term cumulative exposure to natural sunlight, as in the occupational exposure in farmers, fishermen and other outdoor workers,[1] and the incidence of these two malignancies is increasing steadily amongst an ageing fair-skinned Caucasian population. The general public and the media are now showing much more interest and concern about skin cancer and the referral of patients with skin malignancy to dermatology clinics continues to rise.

The management of non-melanoma skin cancer includes local excision or radiotherapy and the prognosis if recognized and treated early is excellent, with cure rates of 96–97%.[2] More difficult and extensive tumours, or recurring tumours may be treated using micrographically controlled surgery.

Cryosurgery for epitheliomas was first reported at the turn of this century by Whitehouse.[3] Despite its initial success it had to be abandoned because of the lack of sophisticated delivery systems of liquid air.

Cryosurgery for malignant tumours of the skin was reintroduced over 17 years ago by Zacarian,[4] and is now well established in the U.S.A. as a treatment for non-melanoma skin cancer, with cure rates of 94–97%.[2] De Lanza, Ralphs and Dawber,[5] reported their results of treatment of Bowen's disease of the skin with cryosurgery in 1980 and have, subsequently, treated other skin epitheliomas with this modality.

I report here my clinical experience and the results of treatment of selected non-melanoma cancers with cryosurgery, between 1981 and 1986.

METHODS

Patients with non-melanoma skin cancer referred to the author at the Department of Dermatology, University Hospital of Wales, were considered for treatment by cryosurgery. Three hundred and ninety five selected skin cancers were treated in 301 patients between 1981 and 1986. These comprised 225 basal cell carcinomas in 174 patients, 128 Bowen's tumours in 85 patients, and 34 squamous cell carcinomas and eight keratoacanthomas (KA) in 42 patients, affecting various sites on the head, neck, trunk and limbs (Table 1).

Tumours with indeterminate margins and those tethered to underlying structures were not selected for cryosurgery. Basal cell carcinomas in the peri-alar crease, the pre-auricular area and the retro-auricular fold were also excluded. Squamous cell carcinomas of the external ear involving underlying cartilage, and deeply invasive squamous cell carcinomas of the lip were not treated with cryosurgery. Sclerosing (morphoeic) tumours were excluded with the exception of two patients who each presented with a single lesion 1 cm and 1·6 cm in size, respectively, on the central right cheek. Exophytic tumours near the inner and outer canthae and in the nasolabial fold were selected for treatment, and tumours were not excluded from treatment with cryosurgery on account of their size.

The clinical diagnosis was confirmed pre-operatively by a skin biopsy. Forty eight (21·3%) of the basal cell carcinomas were superficial BCCs and were found at various sites, including the forehead (16), temple (2), cheek (2), chest (2), back (25), and arm (1). The remaining basal cell tumours were nodular, solid lesions.

Tumour size

Basal cell carcinoma. Of these tumours, 94·2% were 2 cm or smaller across the longest axis of the lesion. Only 13 of the BCCs exceeded this size. Seven of these were nodular tumours on the forehead (2), temple (3), back and shin, and five of these nodular lesions were 2·5 cm in size, the remaining two measuring 4 × 3 cm (back) and 3 × 2 cm (shin). Six superficial basal cell carcinomas were greater than 2 cm; two of these lesions were situated on the chest (both 3 × 1·5 cm), two on the forehead (both 3 × 2 cm) and two were situated on the temple (3 × 2 cm and 5 × 4 cm, respectively).

Bowen's disease. The Bowen's tumours tended to be larger lesions, 20 tumours (15·6%) were larger than 2 cm (Table 2).

Squamous cell carcinoma and keratoacanthoma. Five squamous cell carcinomas (14·7%) were larger than 2 cm (Table 2), but none of the keratoacanthomas exceed 2 cm in diameter.

Treatment method

The tumour was outlined with a 3 mm margin[5] and the area infiltrated with 1% plain lignocaine

TABLE 1. Site and type of tumours treated by cryosurgery

	No. of tumours			
Tumour site	BCC	Bowen's disease	SCC	KA
Scalp	4	2	5	—
Temple	24	6	—	—
Ear helix	1	1	4	—
concha/antihelix	1	—	—	—
lobe	1	—	—	—
mastoid	5	2	—	—
Forehead/eyebrows	47	3	5	3
Upper eyelid	2	—	1	—
Lower eyelid	10	—	—	—
Inner canthus	18	—	1	—
Outer canthus	3	—	—	—
Cheek	18	3	2	—
Nose tip	9	—	1	1
ala	10	1	—	1
bridge and bony side of nose	21	3	4	—
nasolabial fold	5	—	—	—
Upper lip	6	—	—	—
Lower lip	—	2	—	—
Chin	3	—	—	—
Neck	—	1	—	—
Chest	3	—	1	—
Back	27	4	—	—
Genitalia (glans penis)	—	1	—	—
Arm	1	3	—	—
Forearm	1	3	—	—
Hand/fingers	1	19	7	1
Thigh	1	3	—	—
Calf/shin	4	70	3	1
Foot	—	1	—	1

local anaesthetic. Cryotherapy was then carried out using a standard liquid nitrogen open spray technique. Liquid nitrogen was administered with a CryOwen spray gun, using a 'C' tip for tumours not exceeding 1·3 cm diameter and a 'B' tip for tumours up to 2 cm in diameter. Larger lesions were treated by dividing the operative field into areas 1·5–2 cm in diameter and treating each section separately. Bowen's tumours were treated with a single freeze-thaw cycle, with a freeze time of 30 s.[5] The spray tip was held 5–10 mm from the centre of the area to be frozen and the freeze time was measured from the time at which the lateral ice line had formed at the predetermined margin. In the cases of basal cell carcinomas (superficial and nodular) and squamous cell carcinomas and keratoacanthomas, a double 30 s freeze-thaw cycle was employed, and the thaw time between the two freezes was a minimum of 5 min. In some patients the epitheliomas were treated using a cone-spray technique.[6] Temperature monitoring with a thermocouple needle was not routinely employed.

Following treatment, the area was dressed with clobetasol propionate ointment twice daily for 4 days to minimise inflammation.[7] All patients were treated in the Dermatology Treatment

TABLE 2. Sites of Bowen's tumours and squamous cell carcinomas (SCC) exceeding 2 cm

	Size of tumours (cm)	
Tumour site	Bowen's tumours	SCC
Scalp	—	3 × 3; 4 × 5
Retroauricular	2·5 × 1·5	—
Forehead	2·1 × 1·3	—
Temple	2·5 × 2·5; 2·2 × 1·5	—
Cheek	3·5 × 2·5	—
Chest	—	6 × 2
Back	3·5 × 2·0	—
Thigh	2·5 × 1·5	—
Shin	2·5 × 1·5; 4 × 3; 2·5 × 2·0	—
Calf	3·5 × 2·5; 3 × 2·5	3 × 3
	4 × 5; 3 × 3; 3 × 3	—
	4·5 × 4·5; 3·2 × 2·2; 3 × 2	—
Hand (dorsum)	5 × 3; 3 × 3	—
Finger	—	3 × 2

Centre as out-patients, each procedure for a single lesion taking approximately 15 min. Systemic antibiotics were not used. The patients were given an instruction sheet on how to dress the wounds at home.

They were asked to bathe the wound twice daily in tepid water before applying the topical steroid dressing. Paracetamol was recommended, if necessary, for pain relief. The patient returned to the Treatment Centre 5 days later to have the wound inspected and cleaned by the nurse. Povidone-iodine ointment was then used followed by wound lavage each day until healing was complete. All the patients were treated and followed up by the author at 2, 6, 12, 18 and 24 months and then annually thereafter.

RESULTS

Three hundred and ninety five malignancies in 301 patients were treated over a 5-year period, 1981–1986 (Table 1). At the time of writing, these patients had been followed up for periods ranging from 6 months to 5½ years. Twenty four patients were lost to follow-up. These patients were all treated between 1981–1983 and were last examined in 1985, which was between 1½ and 3 years after they had received their treatment. When last seen, these 24 patients were clear of any residual or recurrent skin cancer.

Tumours of the head and neck usually healed within 4 to 6 weeks of treatment and following the resolution of the hypertrophic scar 8 months post-operatively, the cosmetic results were outstanding. Tumours treated on the hands and fingers healed in a similar fashion, with no impairment of digital sensation or joint mobility. Lesions on the calf and shin healed more slowly, healing times being sometimes as long as 6 months.

Tumour recurrences and failed treatment

These results are shown in Table 3.

TABLE 3. Tumour recurrences and failed treatments

Year treated	No. of tumours treated in that year	Tumours which recurred or failed to respond to treatment	Tumour size	Recurrence (Months post-operatively)
1981/82	37	BCC (temple)	1·2 × 1	43
		BCC (chin)	0·7 × 0·7	20
1982/83	57	BCC (forehead)	1·3 × 1·3	10
		BCC (tip of nose)	0·7 × 0·7	18
		BCC (upper lip)	1·2 × 1·2	18
		Bowen's tumour (calf)	2 × 1	6
		SCC (shin)	1·3 × 1·3	13
1983/84	68	SCC (scalp)	4 × 5	Tumour unresponsive
		KA* (tip of nose)	1·5 × 1·5	Tumour unresponsive
1984/85	67	BCC (eyebrow)	1·2 × 1·0	5
1985/86	166	None**		

*KA = keratoacanthoma

**No recurrences at time of writing—follow-up periods 12–24 months.

Basal cell carcinoma

Six out of 225 basal cell carcinomas (2·7%) recurred after a median time of 18 months. One rodent ulcer on the temple recurred 43 months post-operatively. This nodular tumour, 1·2 cm in diameter, had been treated initially by radiotherapy 5 years before cryotherapy. This recurrence was, subsequently, treated by wide excision.

The remaining five recurrent basal cell carcinomas were all small (less than 2 cm), solid protuberant lesions, with clearly defined margins. The recurrences were treated by further cryosurgery. After a median follow-up time of 26 months (15 months to 34 months) these patients remained free of any further recurrences.

Bowen's disease. Out of the 128 Bowen's tumours, one (0·8%) recurred six months after treatment. This histologically confirmed Bowen's tumour was 2 cm in diameter and situated on the right calf. The tumour responded to cryotherapy, but 6 months later a small nodule was palpable at the site. This was excised and was reported as showing islands of squamous cell carcinoma in the dermis with no overlying epidermal change. Excision was complete.

Squamous cell carcinoma and keratoacanthoma. Thirty four squamous cell carcinomas were treated and a recurrence noted in one patient (2·9%). This 71-year-old lady presented with a squamous cell carcinoma on the right shin 1·3 cm in diameter. Histology confirmed the diagnosis pre-operatively and she was treated with a double 30 s freeze-thaw cycle. Healing was complete 4 months later. At follow-up 9 months later, some redness and scaling was noted for the first time in the scar tissue. This was excised and the histology showed changes of an early invasive squamous cell carcinoma. Excision was complete and the scar remained healthy 3½ years later.

Tumours unresponsive to treatment

Two patients failed to respond to cryosurgery. An 80-year-old man presented with a large squamous cell carcinoma on the vertex of the scalp, 4 × 5 cm in area. This was treated with a double 30 s freeze-thaw cycle applied to individual 1·5 cm diameter fields over the surface of the tumour and the surrounding skin. There was little clinical response and therefore the treatment was repeated 2 months later. There was again a poor clinical response and the patient was referred for radiotherapy. He made an uneventful recovery.

A patient with a rapidly enlarging keratoacanthoma 1·5 cm in size, on the tip of the nose, failed to respond to treatment. She was treated with a double 30 s freeze-thaw cycle, but the tumour continued to enlarge causing a great deal of pain. She responded promptly to treatment with radiotherapy, but the resulting scar was complicated by keloid formation, which eventually settled after treatment with flurandrenolone tape.

Side-effects and complications of treatment

Early. Post-operative pain was frequently encountered, usually for the first 2 days. The lesions treated on the forehead and temple sometimes resulted in a throbbing headache, lasting several hours. Soft tissue swelling was often seen, particularly in sites of skin laxity, for example, around the eyes and temple region in the elderly. In some cases the degree of oedema was very marked indeed. The oedema settled within a week or two of treatment. Frank haemorrhage was only seen in three patients at 3, 7 and 20 days, respectively, post-operatively. This complication was treated by inserting an absorbable haemostatic suture into the wound.

Delayed. Hypertrophic scars appeared frequently. Typically they presented as a linear, livid and sometimes telangiectatic thread, 2–3 mm in width, which traversed the diameter of the circular treated field. Initially, it was thought that these lesions were local recurrences; several were biopsied and showed only scar tissue. They were visible most clearly 6 to 8 weeks post-operatively, but they settled and flattened spontaneously at about 8 months post-operatively. Their resolution was slower on the ala nasi.

Milia also developed 6 to 8 weeks post-operatively. They settled spontaneously in most cases and presented only a minor problem, responding to treatment with light curettage if persistent.

Following wound healing, some degree of hypopigmentation was often seen. This was seldom noticeable because many of the patients treated were naturally fair skinned. Patients with a darker complexion had more noticeable hypopigmentation, contrasting with the peripheral skin. Over a period of 3 or more years the areas of hypopigmentation began to darken and the area became less conspicuous.

Notching of the scar due to partial necrosis of the underlying cartilage was seen in only two out of 30 cases of tumours occurring at cartilaginous sites. A 72-year-old lady presented with a nodular basal cell carcinoma affecting the right antihelix, 1·8 cm in diameter. She had a very minor deformity of the scar, having been treated with a double 30 s freeze-thaw cycle. The second patient was a 67-year-old lady with an 0·7 cm nodular rodent ulcer near to the left alar rim. She was treated with a double 30 s freeze-thaw cycle and the cone technique was employed during the second freeze. It is known that the neoprene cone can intensify the freezing of the tissue considerably and at the completion of the second 30 s freeze, the recorded temperature was −60°C.

She was left with some deformity of the left alar rim. This complication has not been seen in the other 11 patients who received cryotherapy to this site. Ectropion and notching of the lower eyelids was not a complication and tenting of the vermilion border of the lips was not seen in any cases treated at these sites.

Cicatricial alopecia of the hair-bearing sites, such as the scalp and temple was often seen. It seldom presented a cosmetic problem, because of the small area affected.

Three unusual complications were seen. A 68-year-old man presented with a Bowen's tumour, 2 cm in diameter, over the anatomical snuff box of the left wrist. He was treated with a single 30 s freeze-thaw cycle, employing the cone technique. Three months later he had a characteristic hypertrophic scar, 20 × 7 mm in area, traversing the treatment field. This scar, subsequently, flattened over the following 7 months, except at one pole where a nodule persisted which became exquisitely tender. This was excised and the histology showed changes of a traumatic neuroma.

A 79-year-old lady presented with a superficial basal cell carcinoma 5 × 4 cm in area on the left temple. The treatment field was divided into quadrants, each treated with a double 30 s freeze-thaw cycle. Three months later a fleshy vascular tumour appeared in the centre of the superio-posterior quadrant. This was excised and the histology showed changes of a pyogenic granuloma.

A 79-year-old lady developed a histologically confirmed squamous cell carcinoma on the vertex of her scalp, 1·5 cm in diameter, 4 years after developing pustular erosive dermatosis of the scalp. The tumour responded well to cryosurgery, but 5 months later a fleshy new growth appeared at the treatment site. This was excised and the histology demonstrated a superficial nodule containing histiocytes, spindle cells and giant cells. The pattern of the cellular infiltrate was that of an atypical fibroxanthoma of Helwig. Immunocytochemical stains for epithelial cells and melanocytes were negative.

DISCUSSION

Carcinoma of the skin is the most common malignancy in man, and early detection and effective treatment gives a high cure rate. The deliberate controlled destruction of tissue by cold injury was first used when suitable refrigerants became available in the 19th century.[8] Cryosurgery for epitheliomas was first reported at the turn of this century by Whitehouse[3] using liquid air. Following the development of more suitable delivery systems, liquid nitrogen was reintroduced as a refrigerant in dermatology to treat neoplastic lesions, such as basal cell and squamous cell carcinomas.[4,9,10]

Successful cryosurgery depends upon an understanding of the effect of freezing living tissue in order to optimise tissue death. Cellular injury following freezing may be brought about by extracellular and intracellular ice formation, hypertonic damage, disruption of phospholipids in cell membranes and cutaneous circulatory changes during freezing and thawing.[8] The critical determinants of the extent of cellular injury in any single freeze-thaw cycle are the rate of freezing, the coldest temperature reached, the duration of freezing and the rate of thawing.[11] Repetition of the freeze-thaw cycle produces greater tissue destruction than a single freeze-thaw cycle.[12]

Opinions differ as to the absolute temperature and its duration, which is necessary to produce tumour necrosis.[13] Temperature recommendations for cutaneous cancers are commonly given as $-25°$C to $-30°$C at the borders and deep margins of the tumours.[6] Measurement of the temperature beneath the skin surface during cryosurgery may be obtained directly by inserting a thermocouple needle into the skin. An indirect measurement of tissue freezing has also been formulated by Torre,[6] who has shown that the extent of the lateral spread of freezing on the skin surface is related mathematically to the depth of the 0°C isotherm. Zacarian[14] has suggested that more precise measurement of the depth of tissue freezing may be achieved by inserting the thermocouple needle through an acrylic jig. Although these electrical instruments will record the temperature of tissue at the needle tip, its relationship to the tissue temperature at the peripheral and deep margin of the tumour is unknown, because it is not possible to judge accurately by clinical methods the position of a thermocouple needle tip in relation to these important anatomical boundaries of the skin tumour. Reliance upon absolute temperature recording could possibly lead to cases of under-treatment or over-treatment. It was for this reason that another treatment regimen for freezing was adopted in the present study.

De Lanza, Ralphs and Dawber[5] standardized a successful treatment for Bowen's disease, employing a single 30 s freeze-thaw cycle to the tumour and its 3 mm margin. The maximum freeze time of 30 s was chosen by these workers because it was then known that the collagen fibre network of the dermis remained largely undamaged by freeze times not exceeding 30 s, thus minimising scarring.[15] Knowing that repeated freeze-thaw cycles are more destructive than a single freeze, a double 30 s freeze-thaw cycle was employed in the present study for more locally invasive tumours such as squamous cell and basal cell carcinomas. A standardized schedule was employed in order to allow more accurate evaluation of the efficacy of cryosurgery. This treatment regimen, although very simple, has now proved to be extremely effective, producing cure rates equal to those in studies employing temperature monitoring.[2] The cosmetic results have been outstanding without compromising the effectiveness of the treatment. Reliance upon temperature monitoring to control the duration of freezing would have often produced freeze times greatly exceeding 30 s and this would inevitably have resulted in extensive tissue necrosis and disfiguring scars.[16] Until imaging techniques can be developed which can localise precisely in three dimensions the margins and depth of a tumour and their proximity to the temperature monitoring apparatus, there seems to be no advantage in recording absolute temperature as a criterion of adequate therapy.

No evidence of cryonecrosis as a long-term sequel to treatment has been seen in the present study, as is seen sometimes, for example, following radiotherapy, nor has this complication been reported elsewhere after over two decades of use of cryotherapy. It would appear that the skin can tolerate this degree of cold injury without any subsequent breakdown. Furthermore, recurrent tumours treated with cryotherapy have healed without complications of tissue breakdown. Tumour recurrences following cryotherapy that have been excised have healed well and have not exhibited the wound breakdown which can be such a difficult surgical problem when attempts are made to excise and repair a tumour recurrence in a radiotherapy scar.

The management of non-melanoma skin cancer includes surgical excision and irradiation, which give composite 5-year cure rates of 95·5% and 94·7%, respectively.[17] Microscopically controlled excision improves the cure rate to 99·1%. Curettage and electrodessication have very limited applications in the management of skin malignancy, producing much lower cure rates of 66%.[18] The cure rates following cryosurgery have been reported to be 96%–97%,[2] which compares extremely well with other treatment modalities.

Some clinicians, however, have reservations about cryosurgery, as the technique does not allow the treated tissue to be submitted for histological examination and evaluation of adequacy of ablation. Similar reservations could be held concerning radiotherapy, but this modality has been used as a very effective treatment for skin cancer for over 50 years. Adequate excision, peripherally and in depth, may be extremely difficult to judge clinically and preparations of tissue for histological examination using the traditional cross sectioning, 'bread loaf' sectioning and peripheral sectioning may fail to detect a tumour extending to the margins of excision.[19] The goal of any treatment must be to eradicate the clinically apparent tumour and its extension into the surrounding normal appearing skin. In the present study, the tumour margin selected was 3 mm, and this margin of clearance was used in the treatment of all the skin cancers irrespective of the tumour pathology. The decision to employ this size of margin was an empirical one based on the earlier encouraging results of de Lanza, Ralphs and Dawber.[5] The asymmetry of skin tumour extension is now, however, well recognized on the basis of intricate mapping of tumour margins by the micrographic technique. It has been shown recently using a micrographic technique that in basal cell carcinomas with diameters less than 2 cm, a minimum margin of 4 mm was necessary to eradicate the tumour totally in 95% of cases.[20] Thus, in a few patients with skin cancer treated by traditional excision and histological control, there will be incomplete excision. However, not all tumours that are incompletely extirpated will recur locally. In a recent 10-year retrosepective study of basal cell carcinoma,[21] 62% of tumours reported as extending to the margins of excision had failed to show any recurrence. The question of selection and adequacy of tumour margins will, therefore, continue to be a controversial one, and is worthy of further controlled studies using different standardized treatment regimens and modalities, particularly when better imaging techniques are available to delineate in three dimensions the size and extension of skin tumours *in vivo*.

It would appear, therefore, that traditional surgery, radiotherapy and cryosurgery, in correctly selected cases offer similar cure rates. Cryosurgery is contraindicated for treatment of skin tumours with indeterminate margins or if tethered to deep structures. Tumours occurring in the nasolabial fold, the peri-alar crease, anterior to the tragus of the ear and the retro-auricular fold may extend deeply and considerably wider than the visible margin, especially morphoeic or sclerosing basal cell carcinomas. Tumours at therse sites are probably best treated by a wide excision and if there is local recurrence, subsequently excised using micrographically controlled surgery. Well demarcated tumours around the eyelids and medial canthus respond well to cryosurgery and the lacrimal apparatus is spared from destruction.

There is no single best method for treatment of non-melanoma skin cancer. Each of the existing modalities has its strengths and weaknesses. The present study has shown that cryosurgery is a safe and effective treatment for non-melanoma skin cancer.

Patients have tolerated this treatment well and explanation beforehand about side-effects of swelling, blistering and pain, and the provision of an information sheet to take home has done much to allay anxiety and fear for the first few days post-operatively. Treatment is performed quickly and cheaply at the out-patient department, in a relaxed atmosphere without the need for general anaesthesia. Cosmetic results of cryosurgery, employing the treatment regimen described here, were outstanding and were superior to modern radiotherapy scars which often show marked atrophy and telangiectasia. The patients were consistently pleased with the results and no patient who subsequently developed skin cancer at other sites refused cryotherapy when this was offered.

Cryosurgery is a relatively new treatment and it will probably remain a controversial modality for some years. Correct selection of patients with skin cancer to be treated by cryosurgery is very important,[22] and will depend on the experience of the operator. With one exception, the recurrences were noted within 18 months of treatment and it is suggested that patients are followed up carefully for at least 2 years post-operatively.

The safety, speed, effectiveness and low cost of cryosurgery and the superior cosmetic results in the hands of an experienced operator are factors that any clinician called upon to treat skin cancer should consider carefully.

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Psoriasis of the scalp treated with Grenz rays or topical corticosteroid combined with Grenz rays. A comparative randomized trial

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SUMMARY

To investigate the effect of Grenz ray therapy alone compared with Grenz rays combined with a topical corticosteroid (betamethasone dipropionate) in psoriasis of the scalp, 40 patients were randomized into two groups. One group received 4 Gy of Grenz rays administered on six occasions at intervals of 1 week and the other group was given the same Grenz ray treatment plus topical corticosteroid. The patients were assessed before and after Grenz ray therapy and were followed-up for 6 months.

Of the 37 patients who completed the trial 16 out of 19 (84%) of the patients in the Grenz ray group, and 13 out of 18 (72%) of the patients in the combination group healed.

The remission time did not differ significantly between the two groups. Of the patients who healed, 5 of 16 patients in the Grenz ray only group and 4 of 13 patients in the combination group remained healed at 6 months.

We conclude that Grenz ray therapy is a useful treatment modality for scalp psoriasis, but that the addition of a topical corticosteroid has only a minor effect.

Psoriasis of the scalp is often difficult to treat successfully. When tar spirit lotions or corticosteroids in gel or lotions are unsuccessful, there are fewer alternative treatment modalities left compared with treatment of psoriasis of other body regions. UVB and PUVA are ineffective owing to the inability of these wavelengths to penetrate hair in sufficient doses. A convenient and effective alternative is Grenz rays. Previously, we have shown that Grenz ray therapy is effective in the treatment of psoriasis of the scalp,[1] and that the combination of Grenz rays and a topical corticosteroid gave faster clearing than the topical corticosteroid alone.[2] The results also indicated a longer remission time with the combination therapy than with Grenz rays alone.[2] To substantiate this, the present study was carried out to compare the effectiveness of Grenz rays alone with Grenz rays plus a topical corticosteroid.

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METHODS

Forty patients, aged 23–76 years with psoriasis lesions on the scalp took part in the study. All lesions were located in hair-bearing areas, and duration of disease was at least 2 years. The patients had been untreated for at least 4 weeks before the start of the study.

The Grenz ray machine factors were 10 kV, 10 mA, half-value layer 0·03 mm Al, half-value-depth in tissue 0·5 mm, focus–skin distance 10 cm.

The patients were divided into two groups according to a randomized predetermined code. One group of 20 patients was treated with Grenz rays, the other with Grenz rays in combination with a topical corticosteroid, betamethasone dipropionate solution (Diproderm®).

Four Gy of Grenz rays were given to each patient in both groups on six occasions at intervals of 1 week. In the combination therapy group betamethasone dipropionate solution was used once a day for 2 weeks, and then twice a week for 4 months.

Clinical evaluation was performed before the Grenz ray therapy and after the 6th treatment. The scalp was examined and erythema, scaling, and distribution (size of affected area) of the psoriatic lesions were graded on a 5 point scale where 0 denoted absence of symptoms and 4 denoted very severe symptoms. The patients used a visual analog scale to indicate itch and these results were converted to a grade on the 5 point scale by the observers. Total score was estimated for each patient by adding the scores for each symptom.

After the last Grenz ray treatment, each patient was told to contact the authors when relapse occurred. Relapse was defined as a need for more active treatment than the usual scalp and hair wash twice a week for the group which had received Grenz rays alone, and for the group which had received the combination treatment relapse was defined as a need for more active treatment than that stated in the betamethasone dipropionate schedule. Each patient was given a letter, giving the definition of relapse, to be returned to the authors at the time of relapse. Patients who did not return these letters were interviewed after 6 months.

RESULTS

The results are summarized in Table 1 and Figure 1. Of the 40 patients who started the trial, three dropped out. One patient in the Grenz ray only group healed after four treatments and withdrew. In the combination group, one patient with a pacemaker withdrew because of fear of adverse effects and one patient healed after six treatments, but did not follow the topical corticosteroid schedule.

The mean severity score for the patients in the Grenz ray only group ($n = 19$) was 9·1 (range 3–12) before and 1·7 (range 0–10) after therapy. The corresponding figures for the combination group ($n = 18$) were 10·4 (range 5–13) before and 1·6 (range 0–8) after treatment. The mean decrease in severity score was 7·4 in the Grenz ray only group and 8·8 in the combination group. This difference was not statistically significant (Wilcoxon's rank sum test).

Sixteen of 19 patients in the Grenz ray only group (84%) healed (total score 0–2) and 13 of 18 (72%) of patients in the combination group. Of the patients who healed 5 of the 16 in the Grenz ray only group (31%) and 4 of the 13 in the combination group (31%) remained healed at 6 months.

DISCUSSION

Psoriasis of the scalp does not differ from psoriatic lesions of the skin, but is often more difficult to treat successfully. In our department, Grenz ray therapy has been used for more than 35 years

TABLE 1. Pre- and post-treatment severity scores in 37 patients with scalp psoriasis treated with Grenz rays alone ($n = 19$) or in combination with topical corticosteroid (betamethasone dipropionate solution, Diproderm®) ($n = 18$)

		No. of patients	
	Severity score	Pre-treatment	Post-treatment
Grenz rays ($n = 19$)	0–2	0	16
	3–5	2	0
	6–8	6	2
	9–11	7	1
	12–16	4	0
Grenz rays + topical steroid ($n = 18$)	0–2	0	13
	3–5	1	1
	6–8	1	4
	9–11	11	0
	12–16	5	0

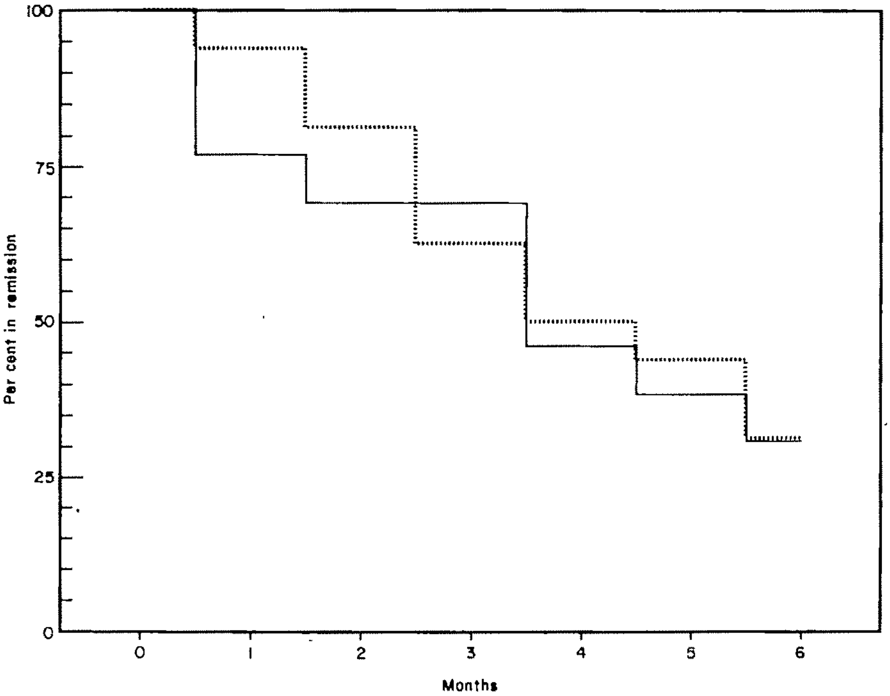

FIG. 1. Duration of remission in 29 patients with scalp psoriasis who showed complete healing after Grenz ray therapy alone (····) ($n = 16$) or Grenz rays in combination with topical corticosteroid (betamethasone dipropionate solution) (——) ($n = 13$).

for the treatment of scalp psoriasis. It has been the clinical impression that it is a valuable alternative when other treatments have failed, and this impression has recently been verified by two studies in which more than 85% of the patients healed almost completely.[1,2] In the present study 84% healed in the Grenz ray only group and 72% healed in the combination group (Grenz rays plus topical corticosteroid). The mean pre-treatment severity score was, however, slightly higher in the combination group than in the Grenz ray only group, which might explain the difference in healing rates.

The remission times for the two groups in the present study did not differ significantly. In the present study 31% of the patients in the Grenz ray only group were healed at 6 months compared with 21% in our first study.[1] Also, in the present study 31% of the patients in the combination group were healed at 6 months compared with 53% in our second study.[2] These differences can be explained by the fact that the groups of patients in the first two studies[1,2] were not matched for severity scores and age.

Many dermatologists have abandoned the use of Grenz ray therapy in the management of benign dermatoses on safety grounds. We believe this is unjustified. In a recent large scale study of the incidence of malignant skin tumours in 14,140 patients who had received Grenz ray treatment for benign skin disorders, it was concluded that even if Grenz ray therapy cannot be excluded as a risk factor in the development of non-melanoma skin tumours, this risk factor is small, if present at all, provided certain recommendations regarding the therapy are followed.[3]

In our opinion Grenz ray therapy should be considered as a useful therapeutic modality for psoriasis of the scalp. In our three studies the healing rates have been 70–85%; 50–70% of the patients have still been healed at 3 months and 20–50% at 6 months after treatment. Grenz ray therapy is also more convenient to administer than other local therapies.

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Two post-operative epidermoid cysts following realignment of the hallux nail

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SUMMARY

We present a case in which two implantation epidermoid cysts appeared following surgical realignment of the hallux nail. Atypical features of this case were the very young age of the patient, the unusual direction of malalignment of the nail and the site and number of lesions.

Post-operative implantation epidermoid cysts occurring in the proximity of scars, is noted in Bunnell's textbook,[1] but escapes mention in many major surgical works. Reports on this topic are scarce and we have found none in the dermatological literature.

CASE REPORT

A 13 month-old boy was referred to us because his mother felt that his great toenails were tender. The least pressure on them made him cry. We recognized bilateral nail malalignment. However, in contrast to the common type, the nails deviated medially and penetrated the red, swollen, inner lateral nail fold.

We decided to treat the nail dysplasia surgically. A crescent shaped wedge resection was carried back to and below the nail bed and the matrix. In contrast to the treatment of the usual type of malalignment, the lateral aspect of the crescent had to be larger than the medial aspect. A small triangular area was also excised at the start of the medial incision line, thereby enabling the whole nail apparatus to be swung over the resected area so that it could be realigned and sutured.

Two months following surgery the child presented with a small, tender tumour about the size of a pea (Fig. 1). It was located at the start of the lateral incision line and there was no inflammation. We diagnosed a post-operative implantation epidermoid cyst, and easily removed the tumour. The pathologist confirmed our views. Six weeks later, the mother returned with her child because of a painful area under the free margin of the nail associated with a small tumour. Surgical removal and subsequent histology showing a cyst filled with orthokeratin and lined by a

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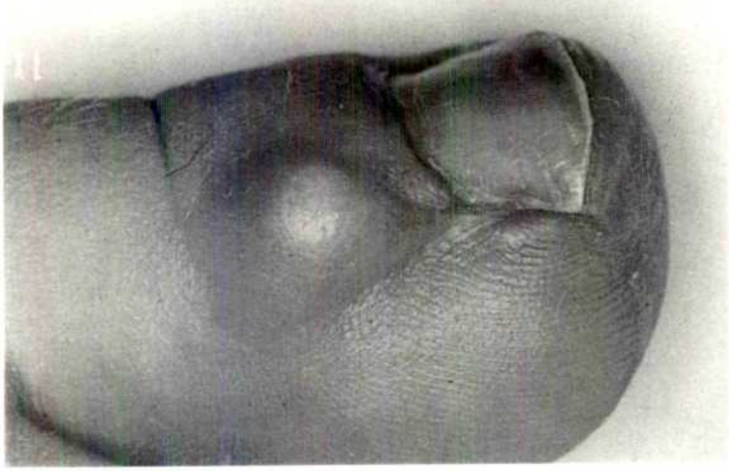

FIGURE 1. Pea size tumour located at the start of the lateral incision line, 2 months after surgery.

thin normal epidermis with a granular layer, confirmed that the lesion was a second tumour similar to the first.

DISCUSSION

The two cysts were sited on or near the incision line. The case showed four atypical features: the very young age of the patient, the unusual orientation of the malaligned nail, and the site and number of lesions.

The condition is extremely rare prior to adolescence and the youngest child on record, to our knowledge, with a typical epidermoid cyst of a distal phalanx, was an 8-year-old girl.[2]

The main characteristic of congenital malalignment of the great toenail is the lateral deviation of the nail plate with respect to the longitudinal axis of the distal phalanx.[3] This is because the nail matrix is set at an angle to the phalangeal axis. In addition to the present patient, we have reported two similar cases, each showing a medial deviation of the great toenail as opposed to the more common lateral deformity.[4]

Epidermoid cysts demonstrate a predilection for the hands. Involvement of the toe is exceptional. We have found only three reported cases in the literature. Roth[5] reported a patient who had pain and tenderness for 5 months in his left hallux, 6 months after a crush injury. Schajowicz *et al.*[6] reported a labourer who substained a contusion of his right hallux 1 year before the first symptoms appeared. In the case reported by Goldsmith,[7] the distal phalanx of the patient's left second toe was swollen with an hippocratic-type deformity, tender but without an inflammatory reaction and there was no evidence of infection. This began 1 year before presentation and progressed very gradually, tenderness setting in some 6 months later.

Epidermoid cysts are always solitary with one reported exception;[5] in this case, the patient presented with squamous cysts in the phalanges of two different fingers.

Epidermoid implantation cysts may be secondary to the trauma of surgical incision. Although not reported in the dermatological literature, their presence has been reported several years after operations on the middle ear.[8] In three cases they had been noted in the temporal region and in one case in the parotid area. The mechanism underlying the formation of these cysts, and those in our patient is probably identical. They were all located on or near surgical scars.

Taking into account the likely process of implantation cyst formation, an important question remains unanswered. Why is it not more common after surgery?

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Painful red hands: a side-effect of leukaemia therapy

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SUMMARY

Five cases of 'painful red hands', following chemotherapy for leukaemia, are reported. All the patients were women who had received treatment with cytosine arabinoside, 6-thioguanine and adriamycin. Several days after courses of chemotherapy, painful erythematous swelling of the palms and soles developed. In all cases the reaction was self-limiting and did not adversely affect their prognosis. Physicians should be aware of this reaction in order to reassure their patients. No treatment is required and the chemotherapy need not be stopped or altered.

Painful red hands, or 'acral erythema', associated with chemotherapy is a recently described[1,2] self-limiting dermatological disorder involving the palms and soles, occurring in patients receiving chemotherapy for leukaemia and other malignancies. It is characterized by the sudden onset of a well-demarcated, acutely tender erythematous or deeply violaceous rash on the palms, fingers and soles. Oedema is often very marked, restricting fine movements of the fingers. The rash may become bullous and then desquamate, but the skin subsequently heals without scarring. Complete resolution usually occurs within 14 days.

Five patients are described who developed this syndrome after chemotherapy for acute myeloblastic leukaemia (AML). All patients received cycles of treatment (CAT) which consists of 5-day courses of cytosine arabinoside 100 mg/m^2 by continuous subcutaneous infusion and 6-thioguanine 100 mg/m^2 orally twice daily, with adriamycin 50 mg/m^2 intravenously given on days 4 and 5 of the cycle. These courses are repeated after intervals of 2 to 3 weeks. All patients were receiving nystatin and chlorhexidine to prevent oral infection.

CASE REPORTS

Case 1

A 40-year-old female presented, in September 1983, with anaemia and thrombocytopenic purpura. Refractory anaemia with excess of blasts was diagnosed on a bone marrow trephine. She was treated with regular blood transfusions, cytosine arabinoside 10 mg subcutaneously

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daily for 6 days a week and retinoic acid 20 mg daily. In December 1984 transformation to AML was diagnosed on bone marrow aspiration. Her treatment was changed to CAT. Six days after completing the third course of CAT she developed an acutely painful, floridly erythematous and violaceous rash involving her palms and soles (Fig. 1), sharply demarcated at the wrists and ankles. Her hands and fingers were extremely oedematous and exquisitely tender when gently compressed. At the time the rash developed, she had received 6 days of intravenous gentamicin and mezlocillin for presumed septicaemia. These antibiotics were continued and intravenous cotrimoxazole added, but she died of fulminant pneumonia 3 days later. Shortly before death there was a decrease in the pain and swelling of her palms, and the rash had subsided.

Case 2

A 49-year-old female, presented in December 1984 with lethargy. AML was diagnosed on bone marrow aspiration, and treatment started with CAT. Six days after the start of the second course and 48 h after the start of the third course she developed painful well demarcated florid palmar and digital erythema and oedema. Over the next 5 days she developed small bullae on the palmar surfaces of the fingers followed by desquamation. Within 10 days of the appearance of the rash there was almost total resolution. The patient was also receiving oral allopurinol, ketoconazole and vitamin E when the rash developed, but these were not stopped. The syndrome did not occur during the initial course of CAT, nor in the subsequent two. Her leukaemia relapsed in October 1985 and she died 14 days later.

Case 3

A 38-year-old female presented in October 1985 with anaemia. AML was diagnosed on bone marrow aspiration and treatment started with CAT. Ten days after completing her second course of CAT, having received gentamicin and mezlocillin for 2 days, she suddenly developed painful erythematous and oedematous palms with clear demarcation at the wrist. She developed bullae on the flexor aspects of her fingers followed by desquamation. Eleven days after the desquamation appeared the changes had fully resolved. Similar changes did not occur after her first course of CAT or after three subsequent courses. In July 1986 she received an uneventful allogenic bone marrow transplant from her sister and was in remission at the time of writing.

Case 4

A 51-year-old female presented in January 1986 with a chest infection. AML was diagnosed on bone marrow aspiration. Twenty four days after her first course of CAT, while on oral amoxycillin, she developed an intense erythema on her palms (Fig. 2) which was clearly demarcated at the wrist and on the sides of her fingers. The erythema was associated with intense pain and some oedema. The palmar erythema faded and was followed by desquamation. Resolution occurred 8 days after the desquamation started. During these changes she developed fingernail dystrophy and lost her nails, which subsequently regrew. She received a further four courses of CAT and underwent an autologous bone marrow transplant without recurrence of her palmar erythema. She was in remission at the time of writing.

Case 5

A 51-year-old female presented in April 1987 with anaemia. AML was diagnosed on bone marrow aspiration, and chemotherapy with CAT was started. Four days after finishing her second course of CAT, while not on any other treatment, she developed a painful erythematous

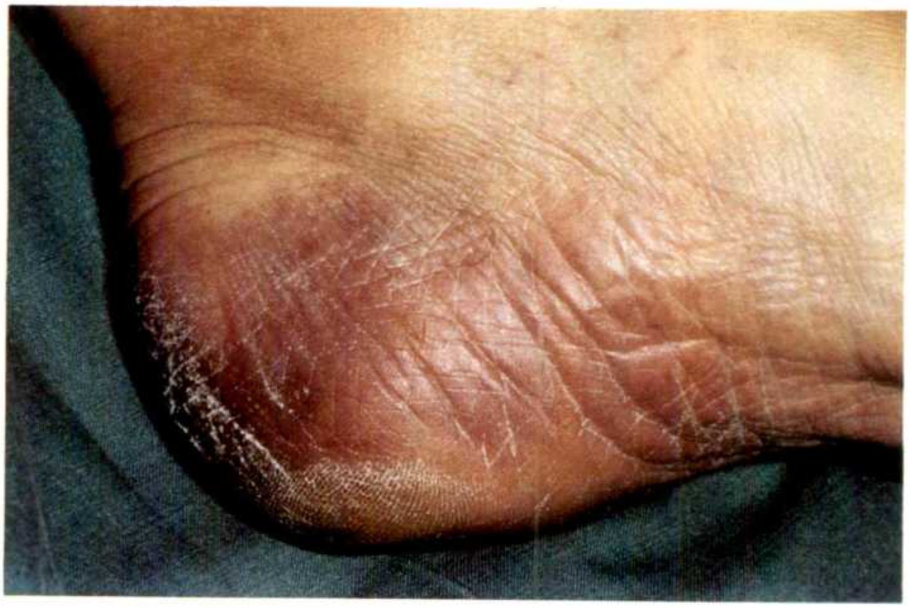

FIGURE 1. Sharply demarcated erythematous, oedematous rash on the sole.

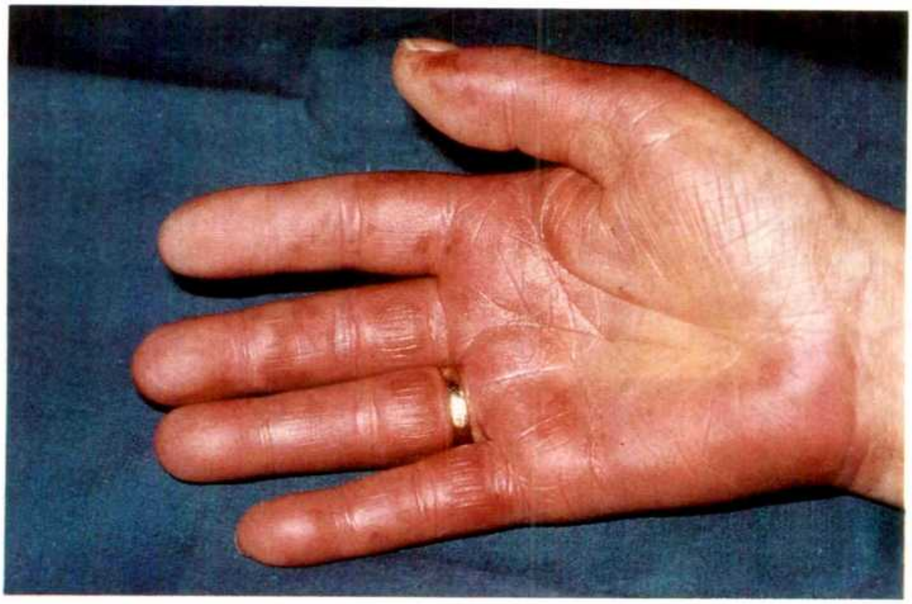

FIGURE 2. Case 4: Intense erythematous to violaceous rash with oedema on the palm sharply demarcated at the wrist. Appearance 3 days after onset.

swelling of the palmar surfaces of both hands. The involved area was well demarcated and localized to her palms and the palmar surfaces of her fingers. The pain and swelling rapidly became so severe that she was unable to flex her fingers. Sections of a skin biopsy from an affected palm, taken 5 days after the onset of the rash, showed basal spongiosis and individual cell necrosis of epidermal cells with orthokeratosis and a mild non-specific inflammatory

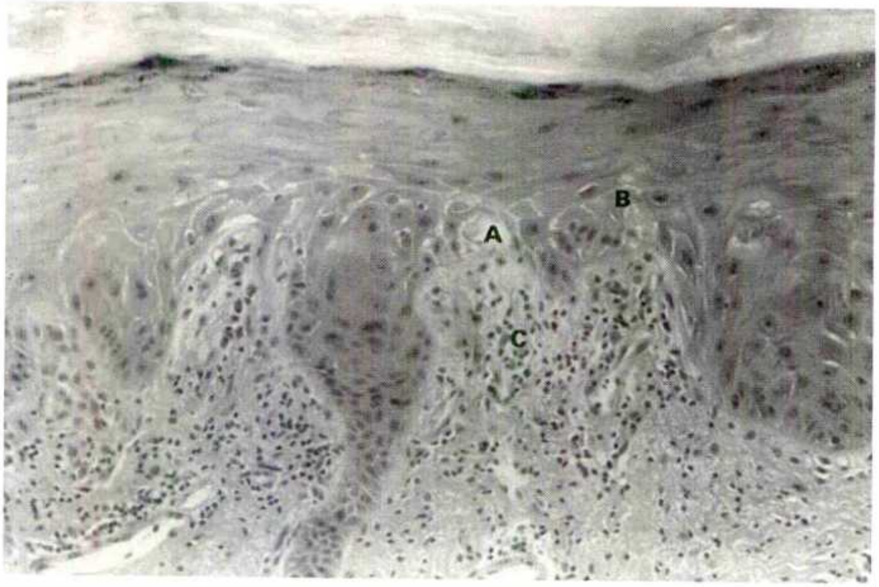

FIGURE 3. Case 5: Skin biopsy from an affected palm, taken 5 days after appearance of the rash, showing basal cell spongiosis (A), individual cell necrosis (B) and a mild non-specific inflammatory reaction in the dermis (C). (Haematoxylin and eosin; original × 400).

response in the superficial dermis (Fig. 3). The epithelium of the sweat ducts showed identical changes to those seen in the epidermal cells. After 8 days the rash began to desquamate and 2 days later had resolved. There was no recurrence after her third course of CAT. She subsequently underwent autologous bone marrow transplantation with carmustine, VP-16 and cyclophosphamide conditioning without further skin problems.

DISCUSSION

Painful red hands in patients undergoing cytotoxic chemotherapy has only recently been described,[1,2] under a variety of names.[1-5] Clinically, the reaction is acute in onset, symmetrical in distribution and sharply demarcated, with erythema as a prominent feature. However, the morphology and distribution of the rash are consistent with either a toxic or an allergic reaction. The histological features lack diagnostic specificity. The changes described here have been noted in previous case reports[2,6] but may also be seen in fixed drug eruptions, morbilliform drug reactions and even graft-versus-host disease. None of our patients had undergone bone marrow transplantation when the syndrome presented. The clinical presentation and histological features of the skin reaction, therefore, do not explain the mechanism. However, the lack of recurrence with further courses of CAT is against either an allergic reaction or a fixed drug eruption.

Chemotherapy for patients with leukaemia and other malignant diseases is frequently given in the form of multi-drug regimens with supportive treatment, including antibiotics, given as indicated. Previous reports of this skin reaction have most frequently involved cytosine arabinoside, given either as high dose single agent therapy[3,6] or in combination with adriamycin and vincristine.[1,2] Skin toxicity appeared to be related to the duration of each course of cytosine arabinoside rather than the peak drug concentration when single high dose cytosine arabinoside was used in refractory leukaemia.[6] Both the clinical and histological findings were identical to those seen in our five cases. We, therefore, suspect that cytosine arabinoside was the agent responsible for the reaction in our cases. Two preparations of cytosine arabinoside, Cytosar® (Upjohn Limited, Crawley, U.K.) and Alexan® (Pfizer Limited, Sandwich, U.K.) were in use interchangeably during the period of this report, but our records do not enable us to distinguish which preparation was used when the rash occurred. As 0·9% preservative free normal saline was used as the diluent in another study[6] with similar skin toxicity we do not think that the diluent plays an important role in causing the reaction.

A similar skin reaction has also been reported after single agent therapy with mercaptopurine[4] and fluorouracil,[5] and also after ProMace,[7] a multi-drug regimen which does not contain cytosine arabinoside. In addition to AML, this syndrome has also been reported to occur after chemotherapy in patients with non-Hodgkin's lymphomas[7] and cancer.[5]

It is important for physicians to be familiar with this reaction in order to reassure their patients, because although dramatic in presentation, it is self-limiting and requires only symptomatic treatment. Painful red hands will not affect prognosis, and subsequent chemotherapy need not be altered.

All the cases have been reported to the Committee for Safety of Medicines.

ACKNOWLEDGMENT

We wish to thank Pfizer Ltd., U.K. and Upjohn Ltd., U.K. for their contributions to the cost of reproducing the colour plates.

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The effect of anti-reactional drugs on complement components in the type II, erythema nodosum leprosum, reaction

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SUMMARY

Seventeen patients with the type II (erythema nodosum leprosum) (ENL) reaction were studied. They received multidrug therapy and also the anti-reactional drugs prednisolone clofazimine or chloroquin, and we measured serum levels of complement components before treatment and after the reaction had subsided.

Factor B was significantly elevated after treatment with each of the three drugs. C_3 levels were significantly increased after treatment, the largest change being in patients treated with clofazimine. In these patients there was also a concomitant decrease in C_{3d} levels. This suggests that clofazimine has complement modulating activity and we would recommend it as the drug of choice in treatment of the ENL reaction.

The clinical features of the type I (lepra) and type II (erythema nodosum leprosum) (ENL) reactions are well known.[1] Several facets of the immunological basis of the type II (ENL) reaction, in particular, remain unknown.[2] Remissions and relapses remain the hallmark of the ENL reaction, despite rapid progress in the treatment of the condition.[1] The advent of steroids and clofamizine was hailed as a great advance, but served only to suppress the symptoms. The ENL reaction may be affected by complement components, which we have recently studied.[3] We report here a study of the effects of anti-reactional drugs (ARD) on the reaction and on complement components.

METHODS

Seventeen patients with the type II (erythema nodosum leprosum) reaction attending the Urban Leprosy Centre, National Leprosy Eradication Programme, took part in the study. The diagnosis was based on detailed clinical examination,[1] bacteriology and histopathology.[4,5]

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For quantitation of complement components 15 ml of blood was collected in a sterile universal container, and allowed to clot at room temperature. The serum was separated and stored at −70°C. We measured serum levels of the classical pathway complement components C_{1q} and C_4 and alternative pathway complement components factor B and C_{3d} and also C_3, a component of both the classical and alternative pathways. C_{1q}, C_4 and factor B were measured by a single radial immunodiffusion technique,[6] using antisera to complement component C_{1q} and factor B (Hoechst Pharmaceuticals, Bombay, India), and C_4 antiserum, from Dakopatts, supplied by J. Mitra, Delhi, India. Kallestads Quantitrol (human serum) was used as a control for C_4 and factor B, and WHO reference serum 67/97 was used as a control for C_{1q}. Complement component C_{3d} was estimated by the method of Perrin *et al.*[7] with minor modifications; 0·2 ml of serum was mixed with 0·2 ml of 22% polyethylene glycol (PEG) and centrifuged. The concentration of C_{3d} in the supernatant was measured using a specific anti-C_{3d} antiserum (Dakopatts, Denmark) employing a radial immunodiffusion technique.[6] C_3 was measured using commercially available Nor-Partigen plates (Behring Werke, AG Marburg, West Germany) containing C_3 specific antiserum.

The patients received multidrug therapy as per the recommendations of the WHO, 1982.[8] This was supplemented by 30–40 mg/day of prednisolone in 10 patients, administered for a minimum of 4 weeks. In eight patients, however, this was continued until the ENL reaction subsided. Clofazimine 200–300 mg/day was administered to four patients for the same period, after which the dose was reduced to 50 mg/day. Chloroquin was used in three patients with a dosage schedule of 450 mg/day for the first week, 300 mg/day for the second and 150 mg/day for the third week. Blood samples were collected 1 week after the clinical subsidence of the reaction for repeat estimation of complement component levels.

Statistical analysis

Levels of complement components before and after treatment were compared using Student's *t*-test.

TABLE 1. Effect of drugs on complement components of the classical pathway in the type II (ENL) reaction. Values are means ± SD

Complement components	Prednisolone	Clofazimine	Chloroquin
C_3 (mg/dl)			
During reaction	83·25 ± 10·70	81·82 ± 17·24	82·66 ± 2·25
After reaction	89·50 ± 16·15	101·87 ± 21·92	95·00 ± 18·02
Percentage change	+7·50 ($P < 0·05$)	+24·5 ($P < 0·05$)	+14·92 ($P < 0·05$)
C_{1q} (units/dl)*			
During reaction	75·45 ± 7·94	79·60 ± 7·60	80·83 ± 3·81
After reaction	75·00 ± 7·16	80·62 ± 1·25	81·66 ± 3·51
Percentage change	−0·005 (NS)	+0·012 (NS)	+0·010 (NS)
C_4 (mg/dl)			
During reaction	30·65 ± 4·30	29·25 ± 3·40	29·16 ± 3·18
After reaction	30·35 ± 5·30	30·67 ± 4·38	29·50 ± 9·26
Percentage change	−0·009 (NS)	+0·46 (NS)	+0·011 (NS)

*Compared with WHO reference serum, taken to contain 100 units/dl.
NS = not significant.

TABLE 2. Effect of drugs on complement components of the alternative pathway in the type II (ENL) reaction. Values are means ± SD

Complement components	Steroids	Clofazimine	Chloroquin
C_3 (mg/dl)			
During reaction	83·25 ± 10·70	81·82 ± 17·24	82·66 ± 2·25
After reaction	89·50 ± 16·15	101·87 ± 21·92	95·00 ± 18·02
Percentage change	+7·50 ($P<0·05$)	+24·5 ($P<0·05$)	+14·92 ($P<0·05$)
Factor B (mg/dl)			
During reaction	21·25 ± 6·16	19·62 ± 4·60	24·33 ± 3·00
After reaction	22·65 ± 5·28	28·87 ± 7·64	28·66 ± 5·50
Percentage change	+6·08 ($P<0·05$)	+44·5 ($P<0·05$)	+17·79 ($P<0·05$)
C_{3d} (units/dl)*			
During reaction	142·97 ± 52·83	157·25 ± 61·09	93·39 ± 37·16
After reaction	156·35 ± 62·52	104·12 ± 47·26	105·40 ± 42·16
Percentage change	+9·35 (NS)	−33·79 ($P<0·05$)	+13·0 (NS)

*Compared with pooled serum controls, taken to contain 100 units/dl.
NS = not significant.

RESULTS

Anti-reactional drugs did not produce significant changes in levels of complement components of the classical pathway in the type II (ENL) reaction (Table 1). However, these drugs affected levels of the central complement component C_3, its breakdown product C_{3d} and factor B (Table 2).

C_3 levels were significantly elevated; clofazimine produced an increase of 24·5% ($P<0·05$), chloroquin 14·9% ($P<0·05$) and prednisolone 7·5% ($P<0·05$). There was a concomitant decrease in the levels of C_{3d} of 33·8% ($P<0·05$) in patients treated with clofazimine and a small non-significant increase in patients treated with chloroquin or prednisolone.

There was a significant increase in levels of factor B with all the drugs; this was maximal with clofazimine (44·5%; $P<0·05$).

DISCUSSION

The type II (ENL) reaction, occurs primarily in lepromatous leprosy. Although its precise nature is still debatable, several factors are known to precipitate the condition.[1] Increased complement turnover and circulating or deposited immune complexes have been reported in this reaction.[9–13]

It is possible that complement activity is unaffected by administration of the anti-reactional drugs prednisolone and chloroquin diphosphate. Thus, these drugs only alleviate the symptoms through their anti-inflammatory action. The effect of these drugs is temporary and subsequent recurrences are frequent and severe.[14]

Our results suggest that clofazimine has complement modulating activity in addition to its bacteriostatic and anti-inflammatory action. We would recommend clofazimine as the drug of choice in the treatment of the ENL reaction. The dosage should be monitored carefully and it may be worthwhile to supplement the therapy with prednisolone, 30–40 mg/day, or an equivalent dose of one of its derivatives. Steroid sparing agents may be used to allow steroids to be tapered off and discontinued.

An alternative treatment might be the removal of the immune complexes by plasmapheresis.

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Recurrent cutaneous Langerhans cell histiocytosis in infancy

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SUMMARY

We report two infants, aged 4 and 9 months, respectively, who had recurrent cutaneous lesions of Langerhans cell histiocytosis confirmed by immunohistochemical and ultrastructural studies. The lesions were confined to the skin and appeared as transient papules on the trunk in both patients. Since neither showed evidence of internal organ involvement or further recurrences of the skin lesions during follow-up periods of 22 and 24 months, respectively, no therapy was given. These cases may be considered as an uncommon benign variant of the Langerhans cell proliferative disorder.

Histiocytosis X was the term proposed by Lichtenstein in 1953[1] for what is now widely accepted as a rare proliferative disorder of Langerhans cells.[2,3] This is the collective designation for the disease spectrum more correctly termed Langerhans cell histiocytosis (LCH),[4] that includes Letterer-Siwe disease, Hand-Schüller-Christian disease, and eosinophilic granuloma, as well as many intermediate and poorly classified forms, which may affect a number of organs including the skin.[5–7] The skin is a frequent site of involvement and may occasionally be the only affected site.[5,8–14]

We report here two infants with recurrent truncal eruptions which proved to be a benign cutaneous variant of LCH. There have been occasional reports of infantile cutaneous LCH with a similar clinical course,[12–14] and clinicians should be aware of the clinical features of this form of the disease.

CASE REPORTS

Case 1

A 9-month-old baby girl was admitted to the Hanyang University Hospital in March 1986, with a recurrent cutaneous eruption. The baby was delivered normally at term, after an uneventful pregnancy and weighed 3100 g. Physical examination at birth showed no evidence of skin

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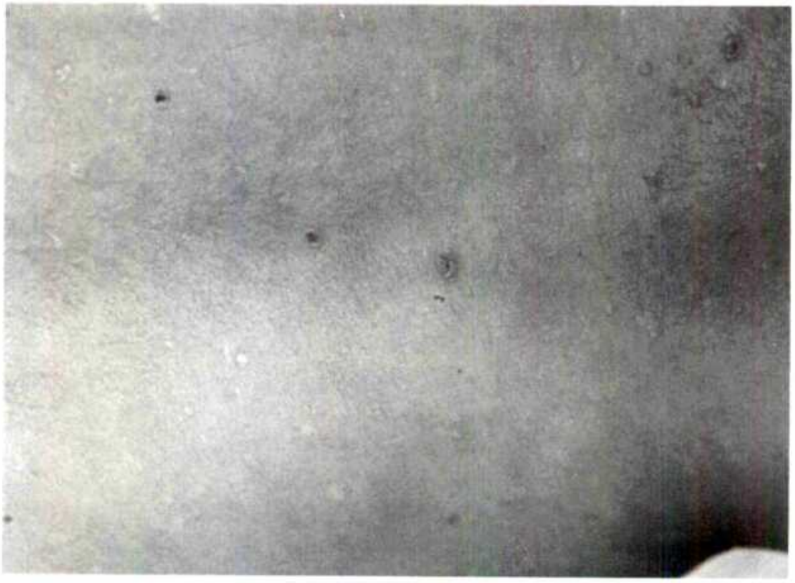

FIGURE 1. Erythematous papules, involuting crusted lesions, and a few hypopigmented spots on the back (Case 1).

disease. When the baby was 4 months old, her parents noted several brown papules on the upper abdomen, which spread over a period of 1 week to involve the lower abdomen and back. The number of lesions had increased to approximately 40. These had involuted within 3 weeks leaving slightly atrophic white spots. The baby remained otherwise in good health. Five months later, 11 days before admission, a further eruption appeared over the abdomen and back; the site and number of lesions being almost the same as previously. There were no apparent precipitating factors.

Physical examination on admission revealed an apparently healthy baby. The liver and spleen were not enlarged, and there was no lymphadenopathy. The skin lesions on the abdomen and back were brown or brownish-red, firm papules, 2 to 5 mm in diameter; some lesions were vesicular with crusts (Fig. 1). Oral lesions were not seen. She was of normal size and developmental stage for her age, and other physical findings were normal.

Histopathological examination of a papular lesion showed focal spongiosis and vacuolar changes in the epidermis with exocytosis of histiocytes. A patchy infiltrate of histiocytes was seen in the papillary and upper dermis. The histiocytes had irregular or kidney shaped nuclei with abundant eosinophilic cytoplasm. A diffuse infiltrate of neutrophils, eosinophils, and lymphocytes was also noted. Reticulin stains showed an increase in reticulin fibres around the tumour cells. A provisional diagnosis of histiocytosis X was made. LCH was confirmed by immunohistochemistry and electron microscopy.

Skeletal and chest X-rays, abdominal ultrasound, and a bone marrow examination were normal. A full blood count, and the results of liver and kidney function tests were all within normal limits. Measurement of serum immunoglobulins showed normal levels. Tests for anti-cytomegalovirus antibody and anti-heterophil antibody were negative. Intradermal skin tests with recall antigens of PPD, tetanus toxoid, and diphtheria toxoid were positive.

No treatment was given, and the baby was followed-up initially at 2-week intervals and later every 2 months. The papular lesions soon became flattened and crusted, and 4 weeks after onset the lesions had completely disappeared. The child remained well during a follow-up period of 22 months without any evidence of cutaneous or visceral disease.

Case 2

A 4-month-old baby girl was referred for an evaluation of a papular skin eruption involving the trunk. The baby was delivered normally at term, after an uneventful pregnancy, with a birth weight of 3400 g. Physical examination at birth was normal, and no skin lesions were noted. When the baby was 40 days old, her parents noted a few brown papules on her abdomen, which increased in number until 2 weeks later there were about 30 papular lesions, scattered over the abdomen and back. The lesions subsided spontaneously over a period of 4 weeks. Three months later, at 4 months of age, the baby showed a recurrence of the skin lesions. At presentation there were about 20 brown or brownish-red, papular lesions on the chest, abdomen, and back, which had developed during the previous 10 days (Fig. 2).

Physical examination showed a healthy, well nourished child. The liver, spleen, and lymph nodes were not enlarged. Cardiac and pulmonary examinations were normal. The skin lesions over the abdomen and back were dark-yellow to brownish-red solid papules, 2 to 6 mm in diameter. The mucous membranes were not affected. Clinically, there was no evidence of internal organ involvement and her size and developmental stage were normal.

A biopsy specimen taken from a brown papule showed an infiltrate composed of large numbers of histiocytes with lymphocytes, eosinophils, neutrophils, and extravasated red blood cells in the papillary and upper dermis. In some areas the infiltrate had invaded the epidermis causing vacuolar changes (Fig. 3). The histiocytes in the infiltrate appeared as large round cells with abundant eosinophilic cytoplasm, and indented or kidney shaped nuclei. Reticulin stains showed an increased density of reticulin fibres around the tumour cells. A diagnosis of

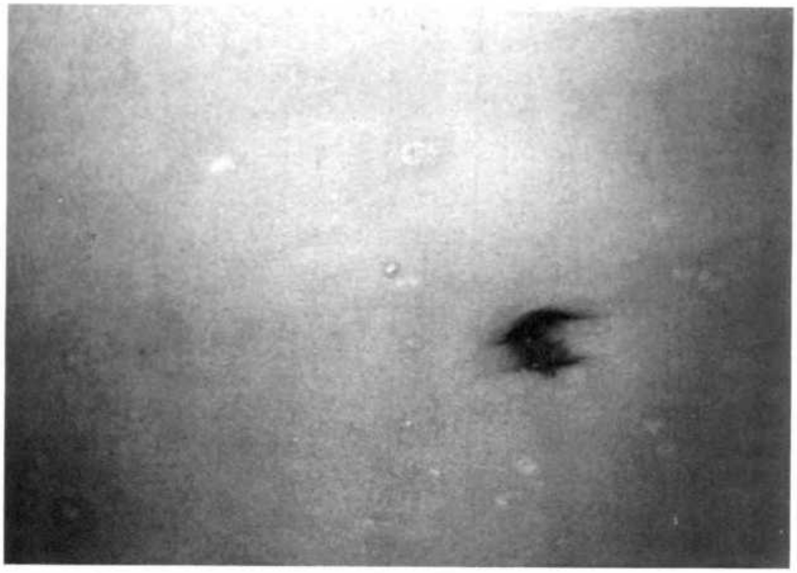

FIGURE 2. Regressing lesions appearing as flat papules with some crusts, and hypopigmented macules left after healing of lesions on the abdomen (Case 2).

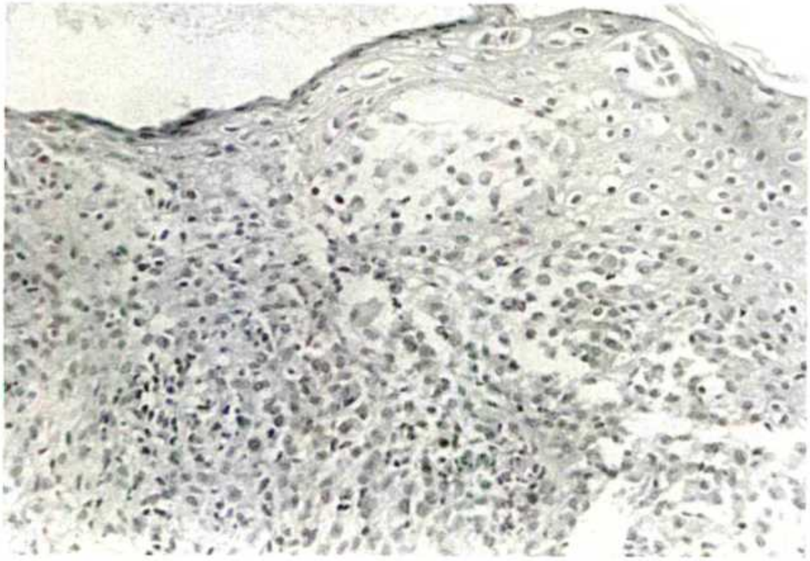

FIGURE 3. An upper dermal infiltrate consisting of loosely aggregated histiocytes and inflammatory cells (H & E, original × 200).

histiocytosis X was made, and LCH was confirmed by immunohistochemical and electron microscopic studies.

Skeletal and chest X-rays, liver and spleen scans, and bone marrow examination were normal. A full blood count, and the results of liver and kidney function tests were all within the normal range. Measurements of serum immunoglobulins were normal. Intradermal skin tests with recall antigens of PPD and trichophytin were positive.

From the 5th day after admission, the skin lesions began to regress, and 4 weeks after the appearance of the first lesion, complete resolution had occurred without treatment, leaving only hypopigmented macules. During the next 24 months of close follow-up, the child remained in good health and no further evidence of the disease was observed.

Immunohistochemical and electron microscopic studies

To identify Langerhans cells in the dermal infiltrate, a biopsy of a brown papular lesion was taken from each patient. Each specimen divided into two parts. One was snap-frozen in liquid nitrogen and stored at −70°C and the other was cut, perpendicular to the epidermis, into small cubes, and immediately fixed in 2·5% buffered glutaraldehyde, processed by standard techniques, and embedded in Epon for electron microscopy.

Sections of snap-frozen tissue from each patient were stained with OKT6 (Ortho Pharmaceutical Corp., Raritan, NJ, U.S.A.) using the indirect immunoperoxidase technique. In each case approximately half of the cells in the dermal infiltrate were stained with OKT6 (Fig. 4). In the overlying epidermis, Langerhans cells were also identified with this antibody.

Ultrathin sections of the Epon embedded tissue were examined under an Hitachi electron microscope (Hitachi Ltd., Tokyo, Japan) at 75 kV. In Case 1, more than one-third of the cells in the dermal infiltrate had the characteristics of Langerhans cells, having abundant cytoplasm containing many Birbeck granules (Fig. 5), and being rich in other organelles including mitochondria, rough endoplasmic reticulum, and Golgi complexes. Some dense bodies with

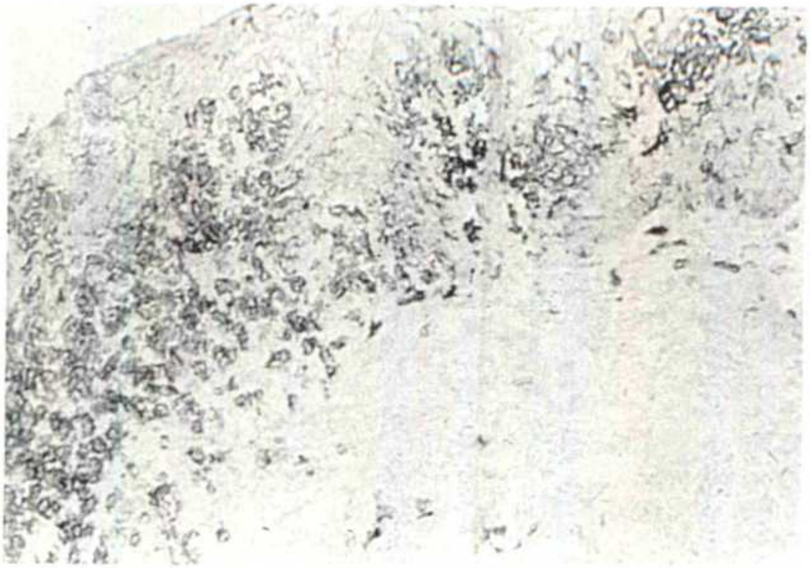

FIGURE 4. Immunoperoxidase staining of lesional skin showing large numbers of OKT6 positive cells in the papillary and upper dermis (original × 200).

concentric laminations were also noted. The nuclei were kidney shaped or lobulated with finely dispersed chromatin. In Case 2, the tumour cells containing typical Birbeck granules constituted about half of the dermal infiltrate. These cells had abundant cytoplasm, large numbers of mitochondria, lysosomes, lipid droplets, and several dense bodies and comma-like bodies. The nuclei were indented with marginated chromatin and prominent nucleoli. These findings confirmed the diagnosis of Langerhans cell histiocytosis in both patients.

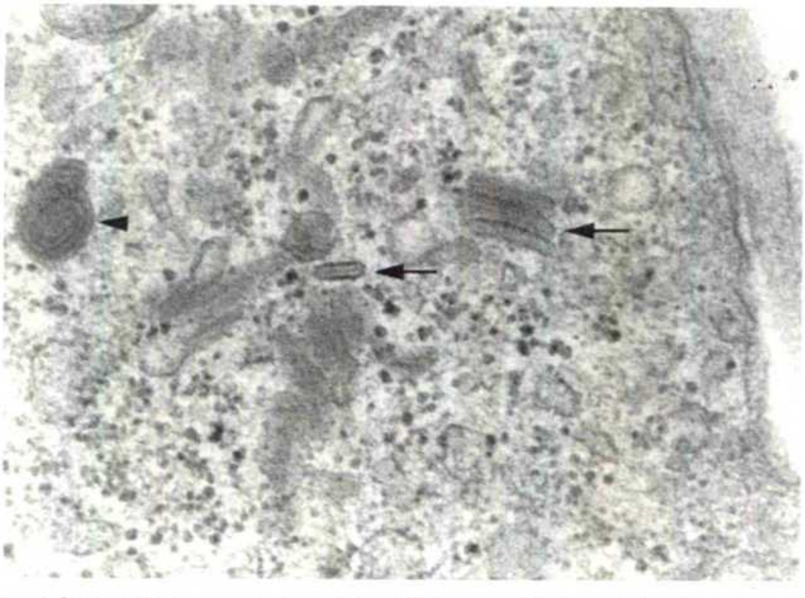

FIGURE 5. A tumour cell containing cytoplasmic Birbeck granules (arrows) and a laminated dense body (arrow head) (original × 40 000).

DISCUSSION

Cutaneous lesions are regarded as a common finding and, not infrequently, the first symptom in Langerhans cell proliferative disorders.[5,7,15] The lesions may have a variety of appearances; most common are brown-to-red papules which may show vesiculation, ulceration, or crusting, or a seborrhoeic, or haemorrhagic appearance.[5,16] Although many reviews stress the association of skin lesions with multi-organ involvement, skin lesions may occur as the sole manifestation of this disease.[8-14]

Our two cases who had non-congenital skin tumours, that consisted of a proliferation of cells bearing the immunohistochemical and ultrastructural markers of Langerhans cells, are exceptional. The lesions were confined to the skin with only one recurrence in each patient. These lesions regressed spontaneously each time, and the patients remained disease free over a 22-month and a 24-month follow-up period, respectively.

In children, the congenital or perinatal form of reticulohistiocytosis, described by Hashimoto *et al.*,[9,11] is best considered as a benign self-healing type of cutaneous LCH. Our cases of the cutaneous form of the disease had similar clinical features to those of perinatal reticulohistiocytosis, but differed in the late onset of the lesions and the tendency of the lesions to recur at the same sites. These two cutaneous forms occurring in infancy appear to represent the benign end of the clinical spectrum of LCH, which is important from a nosological standpoint.

As the cause of this disease remains unclear, the clinical course in an individual patient with LCH is not easy to predict. However, it has been generally considered that a favourable prognosis correlates directly with the age of onset and inversely with the extent of internal organ involvement.[17,18] It may be presumed that if the lesions are confined to the skin the prognosis is generally better than if there is visceral involvement.[10,19] However, some patients who present with skin lesions only may develop multi-system disease within a year,[6,15,16] and clinicians should also recognize that patients with multi-organ involvement rarely go into remission without treatment.[20] Accurate prediction of the clinical course from the morphology and histology of the lesions, and routine immunological tests is generally not possible.[7,20] Therefore, patients should have a thorough clinical examination and laboratory investigation and meticulous follow-up.

The clinical features in our cases suggest that infants with apparently localized LCH, such as that limited to the skin, need careful follow-up only, and therapeutic intervention is not indicated unless there is evidence of extracutaneous dissemination or rapid progression of the disease.

The application of flow cytometric analysis of the DNA from the Langerhans histiocytes in the dermal infiltrate, which tests for the presence of aneuploid histiocyte stem-cell lines with increased proliferative activity, might allow prediction of the biological behaviour of the tumour cells.[21,22]

With an increased awareness of the variability of LCH, atypical presentations can be identified more frequently, and this could expand and refine the clinical categories in this disease. In view of the rarity and benign nature of the recurrent cutaneous form of LCH in infancy, we hope that the report of our two cases may aid in the diagnosis and management of similar cases encountered by other clinicians.

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British Journal of Dermatology (1988) **119**, 267–272.

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Koilonychia in Ladakhis

MADAM, In December 1984 we paid a brief visit to Ladakh in the Northwest Himalaya in order to make preparations for an expedition to study the haemodynamics of the Yak the following summer.[1] At that time we became aware of what seemed to be an unusually high prevalence of a form of koilonychia on the fingers of healthy Ladakhis. In order to verify this chance observation, we obtained permission to study the fingers of men in the Indo-Tibetan Border Police. This force comprises local Ladakhis born and bred at an altitude above 3600 m and also lowlanders from other regions of India who had been transferred to the high altitudes of the northern borders.

The observations were made in Leh (altitude 3800 m) where the 29 Ladakhis and 28 lowlanders who were available on a particular day were paraded for inspection. The average age of the Ladakhis was 34·3 years and that of the lowlanders 33·6 years. Only a small proportion was engaged in police duties; the majority carried out duties ranging from tailor to carpenter.

All 10 finger nails were inspected and a note made of the presence or absence of koilonychia in each nail. The degree of koilonychia was graded as mild (score 1) or severe (score 2). Often the deformity was localized, in which case it was graded as mild. The results were analysed using a chi-squared test. The results are summarized in Table 1. There was a significantly higher score in the Ladakhis ($P < 0{\cdot}001$), the right hand was affected more than the left ($P < 0{\cdot}001$), and there was a significant difference in the incidence on different fingers ($P < 0{\cdot}001$) the first three fingers being affected more frequently than the fourth and fifth. Figure 1 shows the right hand of a Ladakhi with koilonychia affecting the first three fingers.

Four of the Ladakhis and three of the lowlanders had clubbing of the fingers but, of these, only two Ladakhis and one lowlander had mild koilonychia. We looked for, but did not observe, the nail haemorrhages which we found to be prevalent in the Quechua population of the Andes.[2] This may be due to the rather lower altitude of Leh (3800 m as opposed to 4300 m at Cerro de Pasco).

The mean haemoglobin level was 15·9 g/dl in the Ladakhis and 15·5 g/dl in the lowlanders, the rather high values being attributable to the altitude. The mean level of haemoglobin in those Ladakhis showing koilonychia was 15·9 g/dl and in those without koilonychia 16·0 g/dl. Diet was communal and ample and it seems unlikely that there could have been a dietary deficiency of iron or any other factor.

The distribution of the koilonychia, typically affecting the first three fingers of the right hand, is similar to that in iron-deficiency. In iron-deficiency the nails tend to be thin and pliable and thus more readily deformed by the mechanical stresses associated with everyday use. The first three fingers of the dominant hand are those which are most used and the commonest stress would be pressure on the pulp. This will be transmitted mainly to the lateral edges of the nail, pushing them backwards, while the central portion of the nail is protected by the terminal phalanx. All the men were right-handed except one Ladakhi who did not have koilonychia. The nails affected were, therefore, those most subject to lateral backward forces. However, they were neither thinner nor softer than normal. The koilonychia was not associated with duties involving hard manual work. We attempted to judge how much the hands were used by assessing the hardness of the skin of the palms, but this showed no difference between those with and without koilonychia. Altitude did not seem to be a factor. The average length of service at high altitude in the lowlanders was 6·6 years which should have been more than enough for them to acclimatize to the same extent as the native population.

We are left, therefore, with the conclusion that the tendency to koilonychia is a racial characteristic of the Ladakhis who form part of a Tibetan population with a culture and facial appearances quite distinct from the rest of India. We have subsequently had occasion to observe koilonychia also in normal individuals in Tibet.

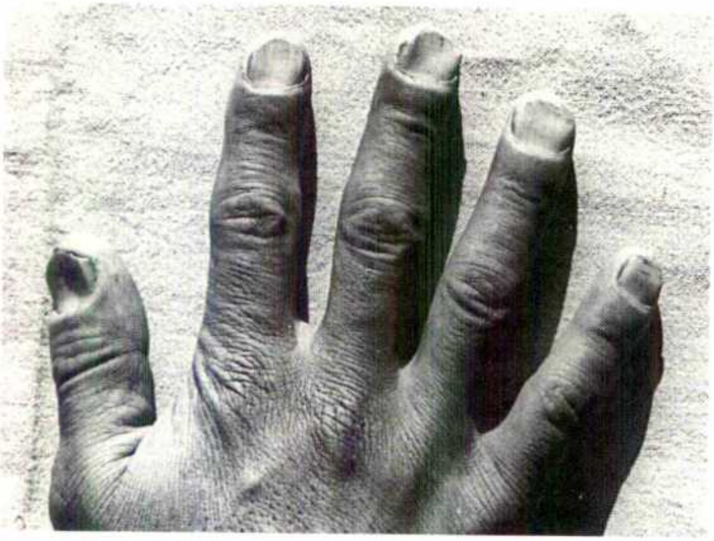

FIGURE 1. Right hand of a Ladakhi showing koilonychia of the first three fingers.

TABLE 1. Total scores for koilonychia on the fingers of Ladakhis and lowlanders

	Fingers of right hand					Fingers of left hand				
	1	2	3	4	5	1	2	3	4	5
Lowlanders	7	7	4	0	0	3	0	0	0	0
Ladakhis	13	17	19	8	4	11	10	10	3	2

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Plasma levels of β_2-microglobulin in psoriasis

MADAM, Elevated plasma levels of β_2-microglobulin (β_2-M) have been reported in patients with some autoimmune disorders, such as SLE,[1,2] Sjögren's syndrome[3,4] and rheumatoid arthritis.[5–7] Numerous factors have been suggested to play a role in the pathogenesis of psoriasis. The presence of immunoglobulin and complement in psoriatic epidermis together with anti-IgG factor[8] suggest that psoriasis may be an autoimmune disease. Therefore, I have measured plasma β_2-M levels in psoriatic patients and the relationship between β_2-M levels and the severity of skin involvement. Twenty-six patients (17 male, nine female; aged 26–82 years), 24 with chronic plaque psoriasis, one with erythrodermic psoriasis and one with psoriatic arthritis were studied. Fifteen patients (seven male, eight female, aged 34–81 years) with other skin diseases (six tinea pedis, four hand eczema, three chronic urticaria, two pustulosis palmo-plantaris) were also studied as controls. Plasma β_2-M levels were determined by using a Phadebas β_2 microtest radioimmunossay kit. Plasma β_2-M levels in the psoriatic patients ranged from 1·1 to 2·9 μg/ml with a mean (± SD) of 1·84 ± 0·59 μg/ml. In the 15 control subjects, β_2-M levels ranged from 0·96 to 2·1 μg/ml with a mean of 1·44 ± 0·36 μg/ml. There was a significant difference between mean β_2-M levels in the psoriatic and control groups ($P < 0{\cdot}05$, Student's t-test). The psoriatic patients were divided into four groups on the basis of the severity of their psoriasis, expressed as percentage body surface involvement. Five had 10% or less body surface involvement (mild), 11 had 10–50% involvement (moderate), six had 50–90% involvement (severe), and four patients had greater than 90% of the body surface involved (very severe). Patients in the very severe group had a mean plasma β_2-M level of 2·25 ± 0·57 μg/ml which was not significantly different from that in the severe group (1·53 ± 0·63 μg/ml), the moderate group (1·80 ± 0·62 μg/ml), and the mild group (1·96 ± 0·37 μg/ml), respectively (Student's t-test).

These results suggest that although plasma levels of β_2-M do not appear be related to the severity of skin involvement, the elevation of β_2-M levels may be important in the modulation of the immune and inflammatory responses in patients with psoriasis.

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Contact urticaria caused by stinging nettles

Madam, The stinging nettles *Urtica urens* and *Urtica dioica* are ubiquitous weeds in Great Britain, and are a frequent cause of non-allergic contact urticaria. Light application of the leaf or stem to the skin results in the rapid development of short-lived wealing accompanied by burning, itching and, frequently, a prolonged tingling sensation which may persist for 12 h or more, long after visible changes have faded. Rook's *Textbook of Dermatology*,[1] in a section on irritant reactions caused by plants, states that 'stinging nettles contain formic acid'. Four other contemporary major textbooks of dermatology make no mention of reactions to stinging nettles at all. Sceptical that formic acid is involved, we were prompted to review the literature.

Both mechanical and pharmacological factors are involved. The nettle's prickly hairs, distributed on both stem and leaf, consist of a minute capillary tube, silicified at its upper end and calcified at its lower end, enclosed at the tip in the shape of the bulb. The latter breaks off after contact with the skin, exposing a fine needle-like point which penetrates the skin, pressure leading to expression of the fluid contents into the dermis.

Although initial studies proposed formic and acetic acid as mediators[2] subsequent investigation confirmed that nettle hairs contain pharmacologically active concentrations of histamine and acetyl choline.[3] They also showed that intradermal co-injection of histamine and acetyl choline in the same concentrations reproduced the nettle sting response. Collier and Chesher[4] identified a third amine, 5-hydroxytryptamine, in nettle hairs. Several investigators have confirmed these pharmacological findings in other members of the nettle family world-wide.[5-8] Saxena *et al.*[6] also showed that the nettle hairs of *Urtica parviflora* contained a histamine-liberating substance. Although histamine, acetyl choline and 5-hydroxytryptamine collectively may well account for the vascular changes and immediate discomfort due to nettle stings, the striking and persistent paraesthesiae remain unexplained and suggest the involvement of hitherto unidentified neurotoxic factors.

In any event, it is highly unlikely that formic acid plays a role in the pathophysiology of this common, but intriguing contact urticaria.

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Failure of isotretinoin to control dermatitis herpetiformis and subcorneal pustular dermatosis

MADAM, In a recent article (*British Journal of Dermatology* 1987; **117**: 297), Lammers and van de Kerkhof have described the inhibitory effects of etretinate on the migration of polymorphonuclear leukocytes (PMNL) into the skin. Another retinoid, isotretinoin (13-cis-retinoic acid), has a number of actions including the inhibition of transcutaneous leukocyte migration in response to activated serum.[1] We have investigated the effect of isotretinoin on the PMNL-dominated cutaneous lesions of dermatitis herpetiformis (DH) and subcorneal pustular dermatosis (SPD).

Five patients with DH (four male, one female, mean age 50·6 years) and one patient with SPD (male, aged 57 years) were studied. The patients, who were all well controlled on dapsone (doses ranging from 100 mg/week to 150 mg/day) were instructed to stop all drug therapy, and after an interval of 24 h began treatment with oral isotretinoin (Roaccutane®) at a dose of 0·5 mg/kg/day. Patients were told they could recommence their dapsone if their eruption was not controlled with isotretinoin.

None of the patients in this study was able to control their skin lesions with isotretinoin. The time taken to relapse varied from 2 to 10 days. Three of the six patients continued taking isotretinoin alone, following the appearance of skin lesions, for periods of 3 to 11 days. All patients reverted to their previous maintenance treatment at the end of the study and regained complete control of their skin lesions.

This study has shown that isotretinoin does not control the skin lesions of DH or SPD. It is possible that a higher dose of the drug may have a beneficial effect, but it has been shown that 0·5 mg/kg/day is effective in suppressing acne in the majority of patients. Although the duration of the study was relatively short, this in itself is unlikely to account for the total lack of response, as isotretinoin has been shown to switch off the PMNL-dominated lesions of pustular psoriasis within 2–5 days.[2] In addition, etretinate is capable of preventing the formation of new lesions in SPD within a few days of commencing treatment.[3] Finally, other drugs that are successfully used in the treatment of DH and SDP such as dapsone, sulphamethoxypyridazine and sulphapyridine also have a rapid onset of action.

The failure to obtain any beneficial response in DH or SPD is more likely to be a reflection of the specificity of action of isotretinoin. It is unlikely that any drug used in the treatment of inflammatory dermatoses is acting by non-specific means on the pathway of leukocyte migration, as this could compromise normal PMNL defence mechanisms. Instead, it is probable that drugs such as isotretinoin, etretinate and dapsone (all of which inhibit PMNL migration in certain clinical and experimental settings) act on specific mediator systems. Thus whilst isotretinoin is effective in blocking migration towards activated serum, it may be unable to influence the response to the (unknown) attractants generated in DH and SPD.

We conclude that isotretinoin is ineffective in the treatment of DH and SPD and that this may be a reflection of the specificity of action of this drug for different inflammatory mediators.

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Commercially available tape for assessing 'skin type'

Madam, British dermatologists may soon encounter patients who have used Sebutape® to establish to what degree they have dry or oily skin. This product has recently become available to the public through retail outlets of 'The Body Shop'. Sebutape® (Cuderm Corp., Dallas, Texas, U.S.A.), a sebum-absorbent tape, has been found to assess reliably the sebum excretion rate in normal individuals,[1] and in seborrhoeic and acne prone patients.[2] Results have shown good accord with those obtained using hexane extraction[1] or the lipometre®.[3]

The Body Shop package comprises four strips of Sebutape® which are placed on the forehead, chin, cheek and side of the nose and left for 1 h. I quote, 'When you peel them off, you have a set of "oil prints" which reveal the differing oil production patterns of your face.' The customer compares the print from each tape with a numbered reference card, enabling a semi-quantitative assessment of each patch from non-oily (1) to oily (5). The implication for customers who 'type' their skin in this way is that it 'enables you to keep track of what is happening to your skin and to take appropriate action'.

There is, at present, no consensus among dermatologists regarding the definition of such 'skin types' and objective data on 'appropriate action' is lacking. However, in view of the growing popularity of The Body Shop and its products with the public,[4] it seems likely that patients who have used Sebutape® may increasingly come to the attention of dermatologists.

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4 Company file, *The Guardian*, 12 January 1988.

Blood levels of cyclosporin

MADAM, I write to apologise to readers for an error which was entirely my fault. I quoted an incorrect high level of cyclosporin required for patients with bullous diseases treated with drug (*British Journal of Dermatology* 1987; **117**: Suppl 32, 113). A new assay kit was introduced, by Sandoz Pharmaceuticals, in the UK in June 1987 and by 1988 no 'old kits' should be available.

The new cyclosporin RIA kit (Sandimmun® Kit) uses monoclonal antibodies. The old technique was based on polyclonal antibodies. Using the new kit, trough whole blood cyclosporin levels must not exceed 225 ng/ml and trough plasma or serum levels must not exceed 75 ng/ml.

If a twice a day regimen is being followed (as it will be in most cases) the sample should be taken just before the next dose, i.e. about 12 h after the last dose.

In practice the trough level is almost always measured in morning clinics, with the patient not taking the morning dose until after the blood sample has been drawn.

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News and Notices

BAD Awards

Dermatology Fellowship
The BAD has once again made available a Dermatology Fellowship. It is envisaged that applicants should be at a relatively late stage in their training (preferably after achieving accreditation) and the Fellowship should be considered as a secondment from an existing Senior Registrar or equivalently graded post in the United Kingdom. The Fellowship can be held only in a university or teaching hospital department in the United Kingdom and is for 1 year only. The maximum value of the Fellowship is £25,000. This amount must include salary and overheads and any technical equipment, materials or support services deemed necessary, and is payable to the supervising institution. For further details and application forms please contact the British Association of Dermatologists, 6 St. Andrew's Place, Regent's Park, London NW1 4LB. Closing date for receipt of completed application forms: 30th November 1988.

Study Fellowships
We are pleased to announce the availability of the BAD Study Fellowship and the Neutrogena Study Fellowship. Both of these awards are intended for Senior Registrars or Consultant Dermatologists who wish to study or participate in research activities in the U.K. for a period of up to 3 months. The value of each award will be up to £3,750, but the exact amount awarded will be at the discretion of a small subcommittee nominated by the Executive Committee of the BAD. For further details and application forms please contact the British Association of Dermatologists, 6 St. Andrew's Place, Regent's Park, London NW1 4LB. Closing date for receipt of completed application forms: 30th November 1988.

ESDR Symposium

The European Society for Dermatological Research and the International Society of Dermatopathology are holding a clinically oriented symposium under the title 'A hard look on collagen related disorders' in Liège, Belgium on 16–18 February, 1989. Guest lectures are planned and free communications are welcome.

Deadline for submission of abstracts, 400 words maximum: 15 October, 1988. For further information, please contact: Dr G.E.Pierard, Service de Dermatopathologie, CHU du Sart Tilman, B-4000 Liège, Belgium

Fourth Inter-Continental Congress of Dermatology

The fourth Inter-Continental Congress of Dermatology will be held in Caracas, Venezuela, 11–14 February, 1989. The Congress, on the theme: Cosmeatrics and Clinical Pharmacology: a global examination of advances in dermatologic therapies, is co-sponsored by the Jefferson Center for International Dermatology of the Thomas Jefferson University, Philadelphia, and the Centro Medico Docente La Trinidad of Caracas, Venezuela. The meeting will also highlight dermatological surgery, laser therapy, ageing of the skin, and international dermatological education.

A portion of the programme will be allotted to free communications. Abstracts of 100 words should be submitted before 15 October, 1988.

Further information available from Lawrence Charles Parish, M.D., Director, Jefferson Center for International Dermatology, 1819 J.F. Kennedy Boulevard, Philadelphia, PA 19103 U.S.A. Telephone (215) 563-8333.

Introductory Course on the Biology of the Skin, Cambridge, December 1988

The annual introductory course 'The Biology of the Skin' will be held in Fitzwilliam College, Cambridge from 12–16 December 1988. This course is primarily for registrars and postgraduate students at an early stage of their training in dermatology but is open to other interested practitioners. Closing date for applications: 25 November 1988. Further information may be obtained from The Conference Secretary (BOS), Postgraduate Medical Centre, Clinical School, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, U.K. Tel. Cambridge (0223) 212216.

British Society for Paediatric Dermatology Annual Meeting

The Annual Meeting of the British Society for Paediatric Dermatology will be held in Liverpool, U.K. on 15 and 16 October 1988. Topics will include: dermatological aspects of child sexual abuse, autoimmune disease in childhood, and the skin and the intestinal tract. The meeting will be held in the Royal Liverpool Hospital, with accommodation at the Atlantic Tower Hotel. Full details and registration forms available from the Local Organizer, Professor C.F.H. Vickers, Department of Dermatology, 2nd Floor UCD Building, Royal Liverpool Hospital, P.O. Box 147, Liverpool L69 3BX, U.K.

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Characterization of specific leukotriene C_4 binding sites on cultured human keratinocytes

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SUMMARY

Peptidoleukotriene C_4 (LTC_4) and leukotriene B_4 (LTB_4) are both suspected of being inflammatory mediators and epidermal mitogenic agents in cutaneous psoriatic lesions. In the present study an LTC_4 specific binding site was characterized in membranes from cultured human keratinocytes (K_d, 8·7 nmol/l; B_{max}, 1·2 pmol/mg protein). In contrast LTB_4 did not show any high affinity binding which could account for its biological effects. These data suggest that LTC_4, unlike LTB_4, acts on epidermal cells through a receptor-mediated mechanism.

Leukotrienes are lipoxygenase products of arachidonic acid which modulate a wide variety of cell functions and seem to play an important role in many inflammatory reactions.[1] For example, the peptidoleukotrienes (leukotrienes C_4, D_4 and E_4) rank amongst the most potent agents that increase vascular permeability.[2,3] In addition, leukotriene B_4 (LTB_4) is one of the most powerful chemotactic factors for neutrophils.[4,5]

Recent attention has been directed towards the formation and role of these eicosanoids in the pathogenesis of psoriasis.[6] In particular, leukotriene C_4 (LTC_4) and LTB_4 are dramatically increased in lesional psoriatic skin.[7,8] They could, in fact, not only be involved in the inflammatory events of the disease, but also in abnormal epidermal growth, since leukotrienes stimulate epidermal proliferation[9] and DNA synthesis in cultured keratinocytes.[10]

In the present study, we tested for a specific high affinity binding site for LTC_4 and LTB_4 on keratinocyte membranes in order to determine whether the biological effect of leukotrienes could be receptor mediated.

METHODS

Reagents

Synthetic leukotrienes C_4, D_4 and E_4 were provided and tested for purity (⩾98% pure) by Dr

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J.P.Girard and Dr J.C.Rossi, C.N.R.S. Montpellier, France. Leukotriene B_4 was a generous gift from Dr J.Rokach, Merck Frosst Canada Inc. [14,15-^{3}H(N)]LTC$_4$ (specific activity 1·44 TBq/mmol) and [14,15-^{3}H(N)]LTB$_4$ (specific activity 1·18 TBq/mmol) were purchased from New England Nuclear (Dupont, France).

Culture of human keratinocytes

Human skin specimens were obtained at plastic surgery. Epidermal cells were isolated by incubating skin samples in trypsin as previously described.[11] The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml) and amphotericin (2·5 μg/ml) at 37°C in a humidified 10% CO_2 atmosphere. Epidermal growth factor (20 μg/ml), cholera toxin (0·84 μg/ml) and hydrocortisone (0·4 μg/ml) were added 24 h after seeding. On average, confluence was achieved within 6 to 10 days. Cells were collected mechanically with a rubber policeman, without enzymatic treatment, in order to avoid the destruction of binding proteins.

[^{3}H]LTC$_4$ binding assays

A cell suspension in Dulbecco's phosphate-buffered saline (pH 7·4) without Ca^{++} and Mg^{++} was sonicated, then homogenized with a Teflon–glass Potter homogenizer. The homogenate was centrifuged at 100,000 ***g*** for 60 min at 4°C and the supernatant was discarded. The pellet was resuspended in 50 mmol/l Tris-HCl buffer (pH 7·4) containing 20 mmol/l $CaCl_2$ and 80 mmol/l L-serine borate complex and stored at − 196°C. This procedure yielded approximately 70 μg of membrane proteins per 10^6 keratinocytes as measured by Lowry's method.[12]

All binding assays were performed at 0°C in 50 mmol/l Tris-HCl buffer (pH7·4), containing 20 mmol/l $CaCl_2$ and 80 mmol/l L-serine borate. Tests were carried out using 50 μg of membrane proteins incubated with [^{3}H]LTC$_4$ (3 nmol/l) in a total volume of 0·12 ml. Following incubation, bound ligand was separated from the unbound fraction by vacuum filtration through Whatman GF/C filters. The filters were washed twice under vacuum in less than 5 s with 5 ml of ice cold buffer. The remaining radioactivity on the filters was measured in 10 ml ACS-II (Amersham, France). Non-specific binding was measured in the presence of a 1000-fold excess of unlabelled ligand.

[^{3}H]LTB$_4$ binding assays

The assays were performed as described above with the following modifications: the 100,000 ***g*** pellet fraction was washed twice in 50 mmol/l Tris-HCl buffer (pH 7·4) containing 20 mmol/l $CaCl_2$ and 30 μmol/l nordihydroguaiaretic acid (NDGA), an inhibitor of 5-lipoxygenase. Binding assays were carried out with 200 μg of membrane proteins in the same buffer.

RESULTS

Time course and reversibility of LTC$_4$ binding

When [^{3}H]LTC$_4$ was incubated with keratinocyte membranes at 0°C, specific binding rapidly reached equilibrium and remained stable for at least 60 min. When an excess of unlabelled LTC$_4$ was added at equilibrium, a rapid decrease in the radioactive complex was observed showing the reversibility of the binding ($t_{\frac{1}{2}}$ < 2 min) (Fig. 1).

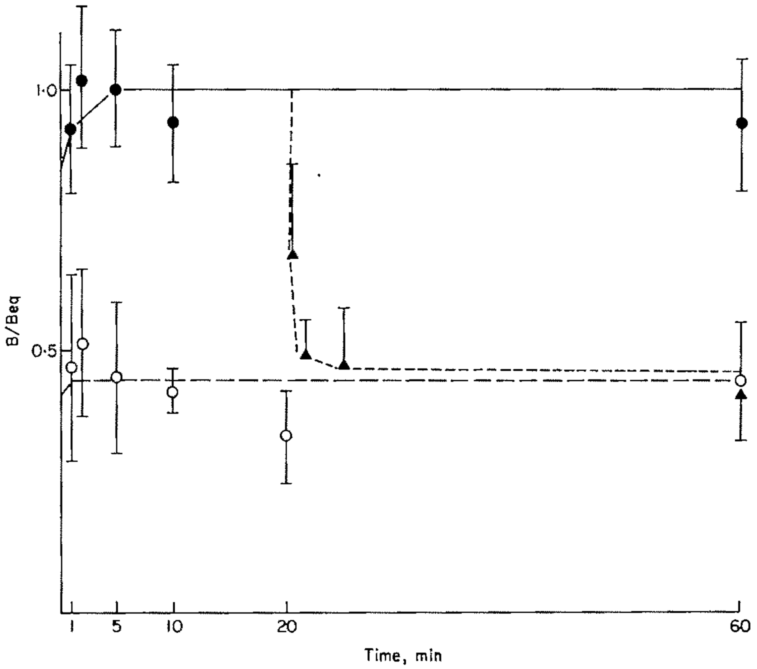

FIGURE 1. $[^3H]LTC_4$ binding to epidermal cell membranes (●), and dissociation (▲) as a function of time. Non-specific binding (○) was measured in the presence of unlabelled LTC_4 (3 μmol/l). At equilibrium, dissociation of the labelled complex was obtained by addition of a large excess of LTC_4 (3 μmol/l). B/B_{eq} = bound radioactivity/bound radioactivity at equilibrium. Points are means ± SD of five different experiments.

Equilibrium parameters of LTC_4 binding

When incubation was carried out for 30 min, specific binding increased linearly with membrane protein concentration. Saturability and affinity of binding were determined by incubating keratinocyte membranes with increasing concentrations of $[^3H]LTC_4$ (0·5–15 nmol/l) (Fig. 2). Scatchard representation of the specific binding resulted in a linear plot corresponding to a single population of binding sites (Fig. 2). The dissociation constant K_d was 8·7 nmol/l and the receptor density B_{max} was 1·2 pmol/mg protein, which corresponded to $5·2 \times 10^4$ sites per cell. It should be noted that non-specific binding was relatively high, possibly due to the use of a crude particulate fraction.

Competition for LTC_4 binding

Incubation of keratinocyte membranes with $[^3H]LTC_4$ (3 nmol/l) in the presence of increasing concentrations of unlabelled LTC_4 resulted in a dose-dependent displacement of $[^3H]LTC_4$ from its specific binding sites. As illustrated in Figure 3, the other leukotrienes (LTB_4, LTD_4 and LTE_4) did not compete for LTC_4 binding sites.

LTB_4 binding

Incubation of keratinocyte membranes with $[^3H]LTB_4$ (0·5–5 nmol/l) failed to demonstrate any specific binding in spite of the special conditions as used by Lin *et al.*,[13] i.e. absence of sodium and washing with NDGA, which allowed characterization of LTB_4 receptor sites in leukocytes.

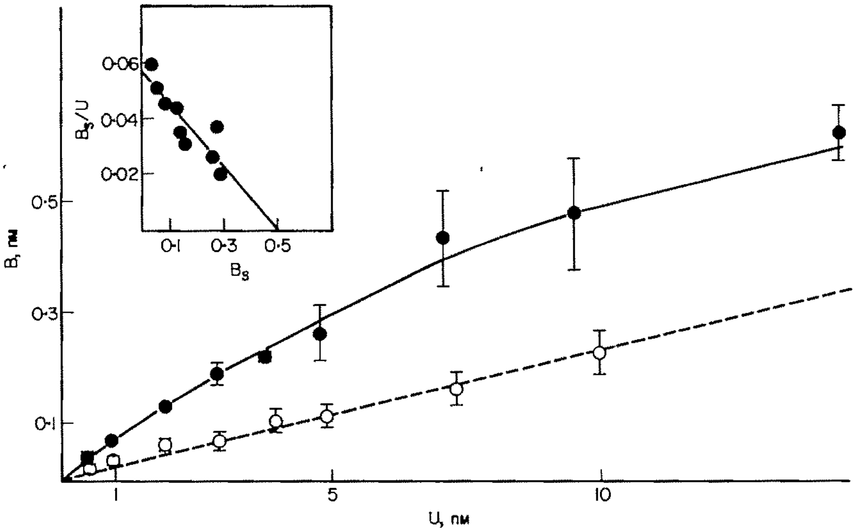

FIGURE 2. Saturation curve of binding of LTC_4. Specific binding (Bs) in the presence (○) or absence (●) of 1000-fold excess of unlabelled ligand. Inset is a Scatchard plot of specific binding (Bs). Bs/U = specific bound fraction/unbound fraction. Points are means ± SD of four different experiments.

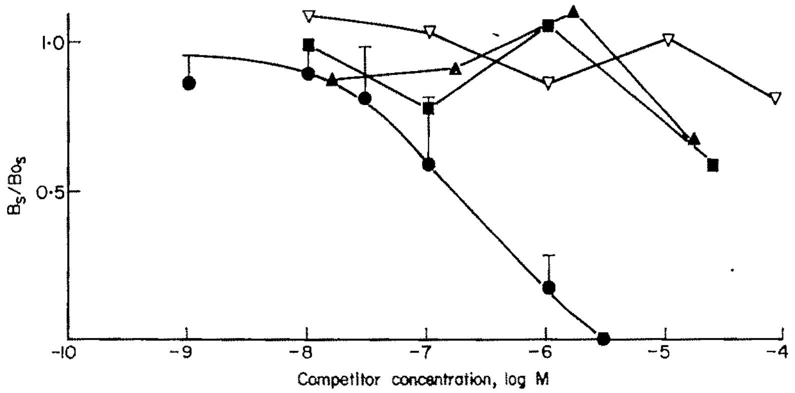

FIGURE 3. $[^3H]LTC_4$ binding to keratinocyte membranes in the presence of unlabelled LTC_4 (●), LTD_4 (▲), LTE_4 (▽) and LTB_4 (■). B_s and Bo_s represent $[^3H]LTC_4$ binding in the presence and absence of competitors, respectively. Points are means of three different experiments, except LTC_4 where they are means ± SD of five different experiments.

DISCUSSION

In the present study a specific binding site for LTC_4 was characterized on cultured human keratinocyte membranes. High affinity sites (K_d 8·7 nmol/l) could be involved in the previously reported proliferative effect of nanomolar concentrations of LTC_4.[10] Similar proliferative activity has also been demonstrated in other epithelial cells and has been correlated with the presence of putative receptor binding sites.[14,15] Other effects of LTC_4, such as smooth muscle contraction, have been linked to high affinity binding in target tissues.[16] The LTC_4 binding site characterized in this study is highly specific, since the relative affinity for LTD_4 is < 1/100, as observed in other cells.[17-20] This implies that LTD_4 has its own receptor sites, since LTD_4 also stimulates DNA synthesis in keratinocytes.[10]

In contrast, however, keratinocytes do not specifically bind LTB_4 in the range of concentrations which increase thymidine incorporation in culture.[10] In spite of the fact that some effects of LTB_4 are triggered by a membrane receptor interaction,[21,22] our results are not in disagreement with the data reported by Kragballe *et al.*[10] The proliferative action of LTB_4 can be related to its calcium ionophoretic capacity,[23] since increased cytoplasmic calcium plays an important role in initiating the process of DNA synthesis and cell division.[24] LTB_4 is 10 times more potent than phosphatidic acid as a calcium ionophore. The latter metabolite has been recognized as a physiological mediator of calcium gating.[25]

The involvement of both peptidoleukotrienes and LTB_4 in psoriasis has recently been reported and provides evidence for the potential usefulness of 5-lipoxygenase inhibitors in the therapy of this disease.[26] In addition, the present demonstration of LTC_4 binding sites on keratinocytes provides a rationale for the use of peptidoleukotriene receptor antagonists[27] in reducing epidermal hyperproliferation.

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PUVA and UVB inhibit the intra-epidermal accumulation of polymorphonuclear leukocytes

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SUMMARY

Healthy volunteers were treated on test areas with UVB irradiation or topical PUVA therapy. The trauma-induced and leukotriene B_4 (LTB_4)-induced intra-epidermal accumulation of polymorphonuclear leukocytes (PMN) was quantified after these treatments, using elastase as a marker enzyme.

Both UVB and PUVA treatment caused a profound inhibition of trauma- and LTB_4-induced PMN accumulation. This observation indicates that reduction of the transepidermal migration of PMN might be part of the mechanism of action of UV therapies in psoriasis. Several possibilities are discussed to explain this hypothesis.

In psoriasis, hyperproliferation and abnormal differentiation of keratinocytes, which lead to scaling, are observed. These alterations in the growth and differentiation of keratinocytes are not specific to psoriasis, however. The phenomenon characteristic of psoriasis is transepidermal migration of PMN, a process which is thought to play a role in the pathogenesis of the disease.

Ultraviolet B (UVB) and psoralen plus ultraviolet A (PUVA) are standard therapies for psoriasis. In both treatments a direct effect on epidermal growth has been demonstrated.[1,2] However, the beneficial effect of light therapies in disorders such as vitiligo, alopecia areata, lichen planus and mycosis fungoides suggests an immunomodulatory effect.[3]

In the present study, we assessed the effects of UVB phototherapy and topical PUVA treatment on the intra-epidermal accumulation of PMN in normal skin following a chemotactic stimulus. The enzyme elastase, which allows accurate quantification of cutaneous PMN, was used as a marker.[4] The chemotactic stimuli were: topical application of leukotriene B_4 (LTB_4), which produces microabscesses of PMN in the epidermis[5] and standardized injury (sellotape stripping), which brings about an endogenous release of chemoattractants and thus causes intra-epidermal accumulation of PMN.[6]

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METHODS

Subjects and experimental schedules

For all experiments the non-sunexposed back skin of 12 healthy Caucasian volunteers, seven males and five females, was used. Six volunteers (mean age 28 years, range 25–35 years, skin types II and III) participated in the experiment with UVB treatment. The other six volunteers (mean age 29 years, range 25–35 years, skin types II and III) participated in the experiment with PUVA treatment.

Experiments lasted 9 days. On days 1,2,4 and 5 irradiation with UVB, PUVA or UVA was given. On day 8 sellotape stripping[6] and LTB_4 application[7] was performed on irradiated areas, areas treated with 8-methoxypsoralen (8-MOP) only and on control (untreated) areas. On days 8 and 9 shave biopsies were taken from the tape-stripped sites and the sites to which LTB_4 had been applied. In addition, biopsies were taken from the irradiated areas and from unexposed areas without previous LTB_4 application or sellotape stripping. Consent of the Medical Ethics Committee was obtained prior to the experiments.

UVB therapy

A skin area measuring 10 × 10 cm was exposed to UVB irradiation, using a Saalmann-SUP (selective ultraviolet phototherapy) lamp, (Gerhard Saalmann, Herford, West Germany). This high pressure mercury vapour lamp emits a spectrum ranging from 290–340 nm, with maximal emission between 310 and 320 nm. The UV radiation flux was 0·58 mW/cm^2, measured at the skin surface with a digital UV meter (Waldmann & Co, Schwenningen, West Germany). Treatment was started at 100% of the individual minimal erythema dose (MED).[8] The mean MED was 51·1 mJ/cm^2 (i.e. 88 s). During consecutive treatments the dosages were increased by 40% if no (or only slight) erythema developed. The preceding dose was repeated if erythema ensued with discomfort. If a subject developed erythema accompanied by pain, irradiation was temporarily discontinued. The mean cumulative dose was 330·6 mJ/cm^2 made up of four irradiations in 1 week. Non-irradiated contralateral 10 × 10 cm skin areas served as controls.

PUVA therapy

In each subject four areas of 10 × 10 cm were delineated for treatment. One area was painted with 5 $\mu l/cm^2$ of 0·15% 8-MOP emulsion (Meladinine®) (Boehringer Ingelheim Haarlem, Holland) and 0·5–1 h later UVA irradiation was given. The second and third areas were treated with 8-MOP alone and UVA alone, respectively, and the fourth area was left untreated. UVA irradiation was given in a Waldmann PUVA 6001 cabin (H. Waldmann & Co, Schwenningen, West Germany). The emission spectrum of the 40 Philips TL 85W/09 lamps was in the range 320–390 nm, with maximum emission at 365 nm. Irradiance measurements were monitored using a built-in PUVA radiometer. Treatment was at 100% of the individual minimal phototoxic dose[9] (mean MPD 0·45 J/cm^2, i.e. 125 s), which was maintained throughout the treatment without any adverse effects.

LTB_4 application, sellotape stripping and biopsies

On day 8 (3 days after the last irradiation), 10 ng aliquots of LTB_4 (Paesel GmbH, Frankfurt, Germany) were applied through glass cylinders onto 5·5 mm circular patches[7] in each of the treated and untreated areas. Application sites were marked with eosin and covered with impermeable dressings (Silverpatch, van der Bend, Brielle, The Netherlands). At the same time, circular regions (15 mm diameter) were stripped[6] by repeated application of sellotape

(30–40 applications) in the treated and untreated areas. Sellotape stripping was discontinued when the whole test site appeared to glisten, and microscopic examination of the sellotape strips revealed no horny cells. Biopsies, using a razor blade in conjunction with a metal guard, were taken from the centre of the stripped test sites 8 h after stripping (time of maximum accumulation of PMN).[6] On day 9 (24 h after LTB_4 application) razor blade biopsies were taken from the LTB_4 treated sites (time of maximum accumulation of PMN).[5] In order to exclude the presence of PMN induced by irradiation, additional biopsies were taken from each of the treated and untreated areas, without LTB_4 application or sellotape stripping.

Analytical procedures

The biopsies (mean fresh weight 3 mg) were processed for elastase measurements as described previously.[4] Briefly, biopsies were washed thoroughly in phosphate buffered saline, weighed, homogenized in a buffer containing cetrimide and centrifuged. Elastase activity was measured in supernatant fractions using the fluorogenic substrate MeOSuc-Ala-Ala-Pro-Val-N-methyl-coumarin. Normal skin contains no elastase activity, but inhibits the PMN enzyme (mean inhibition 12%).[4] For this reason elastase activity was corrected for endogenous inhibition by the inclusion of an internal standard of elastase (equivalent to 500 PMN). PMN were quantified using the value of 0·66 pmol of 4-methyl-7-aminocoumarin released per hour per PMN.[6]

Statistical analysis

The Wilcoxon matched pairs signed ranks test was used.

RESULTS

On day 2 each of the six subjects who received UVB irradiation developed an asymptomatic slight erythema, sharply confined to the treated areas. This was succeeded by a barely perceptible tan or slight hyperpigmentation on day 4 or 5, which lasted for several weeks. In the PUVA treated subjects a slight to moderate asymptomatic pink erythema, again with well defined borders, was observed on day 4, which persisted for several days, without increasing redness. On day 8 a variable degree of hyperpigmentation was seen, lasting for several weeks. No changes were observed in the UVA irradiated, 8-MOP treated or untreated areas.

PMN accumulation 8 h after stripping and 24 h after LTB_4 application is shown in Figures 1 and 2. UVB-irradiated stripped sites and UVB-irradiated LTB_4-treated sites had significantly less PMN accumulation than sites that were only stripped or only treated with LTB_4 (both $P < 0{\cdot}025$) (Fig. 1). A similar response was seen following PUVA treatment. In the PUVA treated areas, PMN accumulation was again significantly inhibited at the stripped sites ($P < 0{\cdot}025$) and at the sites of LTB_4 application ($P < 0{\cdot}05$), compared with the PMN accumulation at the 8-MOP treated, UVA treated and untreated areas (Fig 2). There was no significant difference between these three control sites. In the biopsies taken from treated areas and untreated areas without LTB_4 application or sellotape stripping no PMN accumulation had occurred.

In all biopsies the endogenous inhibition of a standard preparation of PMN elastase was measured. The degree of inhibition in the biopsies of all treatment groups, stimulated with LTB_4 or by sellotape stripping, was below 15% and did not differ significantly from the inhibition of untreated and unchallenged normal skin.

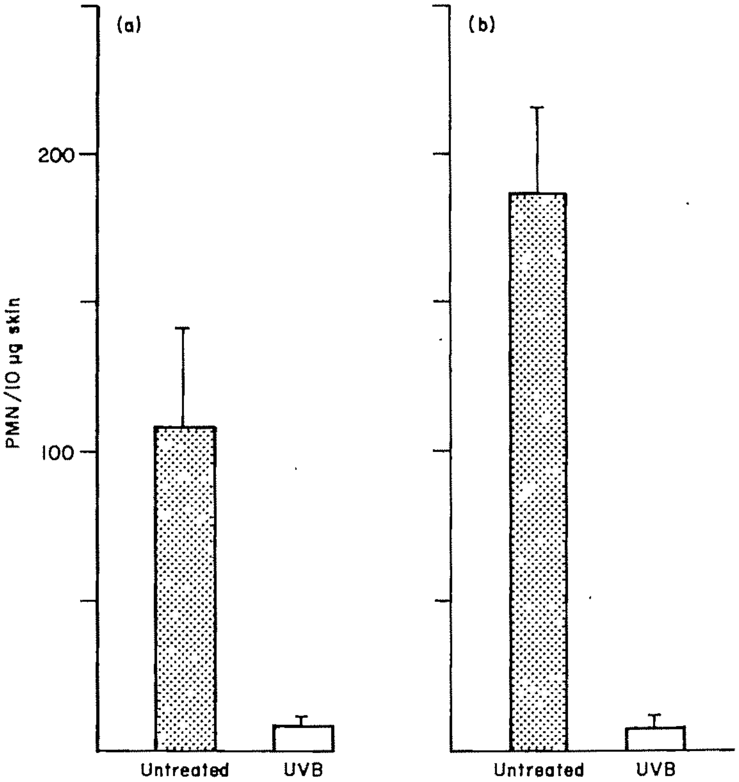

FIGURE 1. Intra-epidermal accumulation of PMN (a) 8 h after sellotape stripping and (b) 24 h after epicutaneous application of 10 ng LTB_4 to untreated and UVB irradiated skin of six normal volunteers. Bars represent means ± SEM.

DISCUSSION

The present study demonstrates that PUVA and UVB phototherapy profoundly suppress both the trauma-induced and the LTB_4-induced intra-epidermal accumulation of PMN. This inhibition was caused by four exposures of normal skin to either UVB or PUVA according to the dosage schedule used in the routine treatment of psoriasis in our department. In the subjects participating in the present study, the clinical responses were limited to asymptomatic slight to moderate erythema, followed by a variable degree of hyperpigmentation. In contrast to PUVA and UVB, UVA irradiation did not affect transepidermal PMN migration, which is in accordance with the substantially higher dosages required for a phototoxic effect.

The transepidermal migration of PMN is an integral function of the circulating blood PMN, the generation of mediators of inflammation and the skin itself with capillaries, dermis and epidermis including facilitating and inhibiting regulatory systems. These different components will be discussed briefly, in order to explain the decreased transepidermal migration of PMN by UV therapies found in the present study.

Both PUVA and UBV may affect the circulating blood PMN. Following whole body PUVA therapy a temporary decrease in circulating blood PMN,[10] and a decrease in chemotactic activity of PMN has been reported.[11] However, other authors could not confirm this.[12] Whole

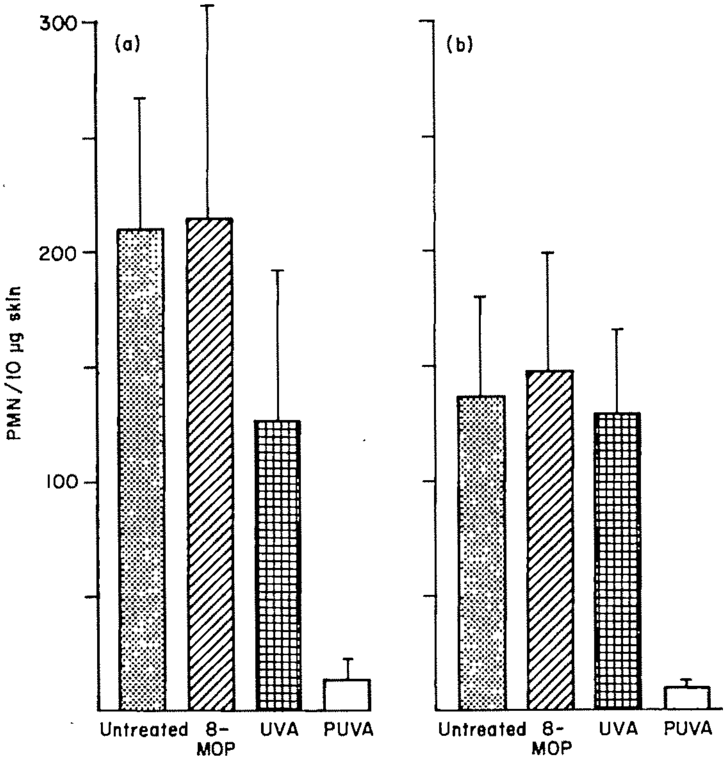

FIGURE 2. Intra-epidermal accumulation of PMN (a) 8 h after sellotape stripping and (b) 24 h after epicutaneous application of 10 ng LTB_4 to irradiated and control skin of six normal volunteers. Bars represent means ± SEM.

body UVB irradiation has been shown to enhance granulocyte function and increase the number of circulating blood PMN.[13] In the present study, trauma and LTB_4 challenge were given at least 3 days after the last UV dose. Therefore, the inhibition of the transepidermal migration of PMN at the UVB and PUVA treated areas cannot be the result of direct interference by UVB or PUVA with the PMN on its way to the epidermis.

The trauma-induced PMN accumulation includes the generation of mediators of inflammation, whereas the LTB_4-induced PMN accumulation bypasses this step. In the PUVA and UVB treated areas the inhibition of PMN accumulation following sellotape stripping was of the same order of magnitude as the inhibition of PMN accumulation following LTB_4 application. If PUVA or UVB inhibit the generation of leukoattractants the inhibition of PMN accumulation following sellotape stripping should have been greater than that following LTB_4 application in the treated areas, as we observed previously following pretreatment with corticosteroids.[14] This earlier observation is in accordance with a direct inhibition of the production of inflammatory eicosanoids via the induction of lipomodulin[15] in addition to interference with the response of PMN to LTB_4.

The movement of the PMN through the skin might be enhanced or inhibited at several levels. Ultraviolet irradiation causes a release of inflammatory eicosanoids, some of which may counteract the LTB_4-induced chemotaxis. Prostaglandin E_2 is released following UVB, but not following UVA irradiation.[16] This prostaglandin has been shown to prevent the accumulation of PMN following intradermal administration of LTB_4.[17] The information with respect to the release of lipoxygenase products following ultraviolet irradiation is rather scanty; however, a five-fold increase of dermal lipoxygenase activity has been shown *in vitro* in the skin of PUVA-treated mice.[18] It is of interest that normal human dermis metabolizes arachidonic acid mainly to 15-hydroxyeicosatetraenoic acid[19] which has recently been shown to inhibit the LTB_4-induced chemotaxis of PMN *in vitro*.[20]

Several authors have reported that the aggregation of PMN and the adhesion of these cells to the endothelial cells are induced by mediators of inflammation, including LTB_4.[21,22] An effect of both UVB and PUVA therapy on the micromorphological appearance of the endothelial cells has been established.[23] Theoretically, chronic damage of the endothelial cells might be accompanied by a reduced adhesion of PMN following standardized injury or epicutaneous application of LTB_4.

In conclusion, the most likely explanations for the UVB and PUVA-induced inhibition of the transepidermal migration of PMN may be the release of eicosanoids, counteracting the effect of LTB_4, or interference at the level of the capillaries. The dramatic reduction of trauma- and LTB_4-induced PMN accumulation following PUVA and UVB seems compatible with their effectiveness as maintenance therapy.[24,25] Further studies to assess the duration of the suppression of PMN migration following treatment with PUVA and UVB would be worthwhile.

Several totally different therapies for psoriasis have been shown to inhibit LTB_4-induced PMN accumulation *in vivo*: methotrexate,[7,26] etretinate,[27] topical corticosteroids,[14] and UVB and PUVA as shown in the present study. Therefore, we may reasonably conclude that reduction of the transepidermal migration of PMN plays a central role in the therapeutic regression of psoriasis lesions.

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Plasma lactoferrin reflects neutrophil activation in psoriasis

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SUMMARY

We used a biotinylated antibody ELISA technique to measure plasma levels of lactoferrin (LF) and the LF content of peripheral blood PMN in 20 patients with psoriasis, 21 with eczema or other inflammatory skin conditions, 19 patients with malignant skin tumours and 20 healthy control individuals.

In psoriasis, plasma LF levels were significantly increased compared with levels in the other skin conditions and in the healthy controls ($P < 0{\cdot}01$). Furthermore, in psoriasis the LF content of circulating PMN was decreased.

These findings provide further evidence that in psoriasis systemic activation ('priming') of circulating PMN may take place.

Migration of polymorphonuclear leukocytes (PMN) into the diseased epidermis is an important feature of psoriasis.[1,2]

Recent work from various laboratories has indicated that in patients with active psoriasis peripheral blood PMN may demonstrate increased functional activities, e.g. adhesion, random motility, chemotactic activity towards various chemotaxins, production of oxygen radicals, and secretion of granule contents.[3–12] On the other hand, several authors failed to show significant differences in PMN taken from psoriatic patients and healthy control individuals.[13,14] The methods used for the purification of cells as well as the variety of tests used to measure PMN functional responses *in vitro* may explain the apparent discrepancies between the results obtained in different studies; different degrees of disease activity may also play a role.[4,6]

In the present study we used plasma lactoferrin (LF) as a marker for activation of circulating PMN. We measured levels of lactoferrin in plasma and PMN from patients with psoriasis, patients with other skin diseases and healthy controls.

METHODS

Details of the patients and controls are given in Table 1. All the patients were hospitalized for their skin disease. All subjects participated in the study after giving their written informed consent.

TABLE 1. Details of subjects studied

	No. of subjects	No. of Males	No. of Females	Mean age (years)
Psoriasis[1]	20	15	5	46
Eczema and others[2]	21	14	7	43
Skin tumours[3]	19	9	10	62
Healthy controls	20	9	11	31

[1] Guttate psoriasis (3), psoriasis vulgaris (10), generalized psoriasis (7).

[2] Atopic dermatitis (5), generalized eczema (8), hand eczema (2), herpes zoster (3), urticaria (3).

[3] Basal cell carcinoma (8), squamous cell carcinoma (5), malignant melanoma (6).

Isolation of peripheral blood PMN was performed as described by Kawohl *et al.*[5] In all subjects the numbers of peripheral blood neutrophils and eosinophils were counted (Table 2). The proportion of eosinophils present in leukocyte isolates from the buffy coat were determined by eosin staining. For determination of cellular lactoferrin levels, neutrophils were sonicated followed by the addition 0·1% w/v cetyltrimethyl ammonium bromide (CTAB). For measurement of lactoferrin levels in plasma and in PMN extracts, a non-competitive avidin biotin ELISA test was used according to a recently described method.[15,16] The most sensitive region of the test was found to be between 1 and 10 ng lactoferrin/well.

The chi-squared test was used for statistical analysis.

Care was taken to exclude possible errors due to blood clotting during the time intervals between blood sampling and the separation of plasma and cells. It has been shown that, even at low temperatures (4°C) peripheral blood PMN may start to secrete LF spontaneously.[17,18] Therefore, in our experiments plasma was separated within 10 min after blood sampling so that spontaneous release of significant amounts of LF *in vitro* could be avoided.

RESULTS

Treatment of isolated peripheral blood PMN by sonication followed by 0·1% CTAB gave a mean LF level of 3·75 μg LF/10^6 PMN. This method proved to be superior to treatment of PMN with Triton X (0·2%) which gave 0·03–0·05 μg LF/10^6 PMN, freeze-thawing (0·03–0·06 μg LF/10^6 PMN) or sonication alone (2·61 μg LF/10^6 PMN).

Since the method used for purification of PMN does not exclude possible contamination with eosinophils[19] which could contain LF, purified eosinophils were examined for the presence of LF. The content of LF in eosinophils from four healthy individuals ranged from 0·008 to 0·033 μg/10^6 eosinophils. This is less than 1% of the LF content recovered from neutrophil preparations and, therefore, was considered negligible.

Plasma LF levels in the patients and controls are shown in Table 2. Plasma LF levels were significantly increased in psoriasis, compared with the control groups. PMN from patients with psoriasis contained significantly less LF than the control groups ($P < 0·001$) (Table 2).

TABLE 2. Lactoferrin (LF) content of plasma and peripheral blood neutrophils from patients with psoriasis and other skin conditions, and healthy controls. Values are means ± SD

	Plasma LF (ng/ml)	Neutrophil LF (μg/10^6 cells)	Neutrophil counts (cells per μl^3)	$\frac{\text{Plasma LF}}{\text{Neutrophil LF}}$
Psoriasis	125 ± 47*	2·39 ± 0·81**	5167 ± 1782	64·3 ± 40·9
Eczema and others	88 ± 34	2·96 ± 1·38	5188 ± 2239	32·5 ± 12·4
Skin tumours	91 ± 32	3·31 ± 0·74	5016 ± 1801	28·9 ± 12·3
Healthy controls	90 ± 34	3·41 ± 1·01	3755 ± 1034	27·1 ± 9·8

* $P<0{\cdot}01$, ** $P<0{\cdot}001$ compared with controls

TABLE 3. Plasma lactoferrin (LF) levels in psoriatic patients and control individuals in relation to age

	Psoriasis patients		Controls		
Age (years)	*n*	Plasma LF ng/ml	*n*	Plasma LF ng/ml	*P*
Below 31	6	151·8 ± 54·9	8	90·8 ± 34·8	< 0·05
Over 50	9	105·0 ± 37·7	9	86·1 ± 37·9	NS

Values are means ± SD
NS = not significant

Since levels of plasma LF may be related to the number of circulating PMN, the ratio of plasma LF: neutrophil LF content was calculated. As can be seen in Table 2 the ratio of plasma LF to neutrophil LF content was more than doubled in psoriasis. This demonstrates that in psoriasis patients plasma LF concentration is inversely related to the LF content of peripheral blood PMN.

Comparison of plasma LF levels in psoriatic patients under the age of 31 years and those older than 50 years showed that the young psoriatic patients tended to have higher LF levels than the older patients. There was no significant difference between LF levels in patients over 50 years and levels in age matched controls. However, young psoriatic patients had significantly higher plasma LF levels than age matched controls (Table 3).

DISCUSSION

The results of this study show that plasma lactoferrin levels were higher in patients with psoriasis than in patients with non-psoriatic inflammatory dermatoses, neoplastic dermatoses and healthy controls.

In parallel to the increased plasma LF levels peripheral blood neutrophils from psoriasis patients were found to contain lower levels of LF, suggesting secretion into the extracellular surroundings.

Lactoferrin is present within the specific granules of PMN.[20] It is synthesized during the promyelocytic phase of cell maturation and synthesis comes to an end at the metamyelocytic stage so that peripheral blood PMN are unable to synthesize more LF once it has been secreted.[21]

In a number of studies, plasma LF proved to be a useful marker for neutrophil activation.[2,13,14,22] Van Snick *et al.*[23] and Leffell and Spitznagel[20] demonstrated secretion of LF during phagocytosis. Furthermore, in acute bacterial infections the LF content of peripheral blood PMN was found to be decreased.[24,25] Moreover in acute bacterial infections, Hansen *et al.*[24] found that while PMN levels of LF were low plasma levels of LF were increased. Our present results suggest that in psoriasis, as in bacterial infections, increased plasma LF levels together with lowered LF content in PMN reflect activation of circulating neutrophils.

These changes were not found in any of the other skin conditions studied. Therefore, secretion of LF by circulating PMN appears to be disease-related and resembles enhanced responsiveness of neutrophils following *in vivo* exposure to chemotaxins. *In vitro* experiments have shown that enhanced PMN responsiveness was seen when the cells were preincubated ('primed') with known activators such as lipopolysaccharides, chemotactic factors, cytokines or arachidonic acid derivatives at low concentrations.[26] Thus, in psoriasis, increased PMN functional activity including secretion of LF is likely to reflect release of systemic secretagogues priming circulating PMN.

Lundin *et al*[7] recently reported increased serum concentrations of LF in psoriasis compared with healthy controls. An inherent and increased tendency of PMN to secrete granule proteins was suggested. Since, however, increased PMN responsiveness in psoriasis, as well as in infectious conditions,[24] is transient and becomes normal during remission,[6] systemic effects related to disease activity appear more likely.

Our observation that young psoriasis patients had higher plasma LF levels may be related to disease type e.g. early onset versus late onset, as previously described.[27]

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Calcium/calmodulin regulation of the proliferation of human epidermal keratinocytes, dermal fibroblasts and mouse B16 melanoma cells in culture

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SUMMARY

We have investigated the relationship between extracellular calcium, intracellular calmodulin and proliferation in normal keratinocytes. Keratinocyte proliferation and its sensitivity to calmodulin antagonists was compared with that of normal human dermal fibroblasts and neoplastic mouse B16 melanoma cells.

Keratinocytes were similar to fibroblasts in showing reduced proliferation in low (0·15 mM) calcium medium and unlike B16 cells which continued to proliferate until calcium was reduced to submicromolar levels. Intracellular calmodulin was significantly higher in rapidly dividing keratinocytes in normal (1·15 mM) calcium medium than in slower dividing cells in low (0·15 mM) calcium. Fibroblasts and B16 cells maintained similar calmodulin levels in both low and normal calcium media. Calmodulin antagonists inhibited proliferation of all three cell types equally. Thus, keratinocyte calmodulin seems related to the proliferative state of the cell (unlike fibroblast calmodulin) and calmodulin antagonists may be of use in controlling the hyperproliferation of the psoriatic epidermis.

We[1] and other workers[2–4] have reported epidermal calmodulin to be elevated in psoriasis. Calmodulin mediates many of the intracellular actions of calcium, including cell proliferation[5] and epidermal calmodulin levels decrease towards the normal range following clearance of psoriasis with treatment.[6] Furthermore, one of the drugs (anthralin) used topically in the treatment of psoriasis has the ability to inhibit calmodulin activity.[7] However, rapidly dividing

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and transformed cells do not necessarily contain higher levels of calmodulin[8,9] and we were unable to mimic this elevation in epidermal calmodulin by inducing normal epidermal cells to divide using sellotape stripping.[1] This raises the question of whether the elevated calmodulin level in psoriasis is related to the hyperproliferative state of the epidermis.

Thus, we have investigated further the relationship between epidermal keratinocyte calmodulin levels and the rate of proliferation of these cells. In particular, we wished to examine whether keratinocyte proliferation could be reduced by the use of calmodulin antagonists.

Cultured normal keratinocytes were used (psoriatic keratinocytes are not distinguishable from normal keratinocytes in culture, both proliferating rapidly[10]), and their rate of proliferation manipulated by altering the concentration of extracellular calcium. Non-transformed cells will usually cease division when the level of calcium in the medium is low (less than 0·2 mM), but neoplastic cells will continue to divide at much lower extracellular calcium concentrations.[11] As workers are divided on the effects of low calcium medium on keratinocyte growth[12–14] and little is known about the effect of extracellular calcium on intracellular calmodulin concentration, we have compared the behaviour of keratinocytes in culture with that of normal and neoplastic cells, using human dermal fibroblasts and mouse B16 melanoma cells respectively. We then compared the ability of an established calmodulin antagonist W7[15] to inhibit the proliferation of rapidly dividing keratinocytes, fibroblasts and melanoma cells.

METHODS

Materials

[^{3}H]-thymidine and calmodulin radioimmunoassay kits were obtained from Amersham International Limited, Bucks, U.K. Medium-199, modified Eagle's minimal essential medium (MEM) for suspension cultures, phosphate-buffered saline (PBS), foetal calf serum (FCS), glutamine, penicillin, streptomycin and 3T3 fibroblasts were obtained from Flow Laboratories Limited, Herts, U.K. Tissue culture plastics were obtained from Becton Dickinson (U.K.) Ltd, Oxford, U.K. EGF, hydrocortisone, dispase, insulin and Chelex 100 were obtained from Sigma Chemical Company, Poole, Dorset, U.K. The calmodulin antagonists N-(6-aminohexyl)-5-chloro-1-naphthalene sulphonamide (W7) and an analogue of W7, N-(6-aminoctyl)-5-iodo-1-naphthalene (iodo-C_8-W7) were a gift from Dr. M. Blackburn, Department of Chemistry, Sheffield University. Drugs were initially made up at 10 mM concentration in dimethylsulphoxide (DMSO) and then diluted in PBS. Appropriate DMSO controls (1% maximum) were included in all experiments.

Cell cultures

B16 melanoma cells were derived from tumours serially transplanted in C57BL mice as previously described.[15] Primary cell cultures were grown in medium-199 with 20% FCS, penicillin (100 IU/ml) and streptomycin (100 μg/ml). Cells were used in their first passage.

Human fibroblasts were obtained by explant culture of dermal pieces of normal foreskin (donor age range 8 months to 3 years) and grown in Medium-199 as for B16 cells.

Human keratinocytes were obtained from foreskins after overnight incubation with dispase at 4°C. The epidermis was separated from the dermis and incubated with 0·05% trypsin, 0·01% EDTA for 5–10 min at 37°C. Cell suspensions were obtained by pipetting and filtration through a double layer of sterile gauze. Cells were cultured in medium -199 with 10% FCS, penicillin (100 IU/ml), streptomycin (50 μg/ml), EGF (10 ng/ml), insulin (4 μg/ml) and hydrocortisone (0·4 μg/ml). Cells were plated on a layer of mitomycin-treated 3T3 fibroblasts (Flow

Laboratories) by the method of Rheinwald and Green[16] for cell counts (after removing 3T3 cells with 0·02% EDTA) and on collagen coated dishes according to the method of Hawley-Nelson *et al.*[13] for cell cycle analysis and intracellular calmodulin measurements.

Manipulation of extracellular calcium

All cells were plated in medium-199 initially, fibroblasts and B16 cells for 24 h and keratinocytes for 7 days and then incubated in Eagle's MEM low calcium medium (found to contain 0·1 mM calcium) with 10% chelex-treated FCS. Treatment with chelex, a 30% slurry of chelex in FCS stirred at 4°C for 24 h, reduced FCS calcium to 0·05 mM as determined using a colorimetric assay for calcium.[17] The total calcium concentration of the medium plus 10% FCS was found to be 0·15 mM. Further calcium chloride was added to the medium to increase extracellular calcium (up to 2·15 mM). The chelating agent ethylene glutamine tetracetic acid (EGTA) was added to decrease extracellular calcium, on the basis of the dissociation constants of Caldwell[18] for Ca^{2+}, Mg^{2+} and EGTA at pH 7·4. All incubations were performed in triplicate.

Measurement of cell proliferation

Cell proliferation was determined in three ways, by counting trypsinized cell suspensions using a haemocytometer, by $[^3H]$ thymidine incorporation into DNA, using a 6 h pulse with $[^3H]$ thymidine, as described previously,[15] and by cell cycle analysis using a fluorescence activated cell sorter (FACS) (Becton-Dickinson). In the latter, cellular DNA was labelled with mithramycin:ethidium bromide 1:1[19] and the distribution of cells in G1, G2 and S phase determined.

Calmodulin antagonists

Cells were incubated with W7 or iodo-C_8-W7 at concentrations between 1 and 100 μM for 3 days, after which the effects of the drugs on proliferation were determined by cell counting and cell cycle analysis. Drugs were added 24 h after plating for fibroblasts and B16 cells and 3 days after plating for keratinocytes.

TABLE 1. Effect of extracellular calcium concentration on fibroblast and B16 melanoma proliferation in culture

Index of proliferation	Experiment no.	Concentration of calcium giving 50% inhibition of cell growth		
		Dermal fibroblasts	B16 melanoma	
$[^3H]$ thymidine uptake	1	2×10^{-4}	$3·1 \times 10^{-7}$	
	2	$2·7 \times 10^{-4}$	$3·1 \times 10^{-7}$	
Cell number	1	1×10^{-4}	1×10^{-7}	
	2	5×10^{-4}	$1·8 \times 10^{-7}$	
	3	$0·4 \times 10^{-4}$	$3·6 \times 10^{-7}$	
Mean ± SEM ($n=5$)		$2·2 \pm 0·7 \times 10^{-4}$	$2·5 \pm 1·0 \times 10^{-7}$	$P<0·001$

Cells were incubated for 3 days in medium containing 5×10^{-8} M to $2·15 \times 10^{-3}$ M extracellular calcium. The greatest rate of cell proliferation in each experiment was taken as 100% for the purposes of calculating the extracellular calcium concentration producing 50% inhibition (IC_{50}).

TABLE 2. Growth of normal human keratinocytes in low (0·15 mM) and normal (1·15 mM) extracellular calcium

Experiment no.	% of cells in S phase Low calcium (0·15 mM)	Normal calcium (1·15 mM)	*P**
1	9·3 ± 1·2	19·0 ± 1·4	< 0·001
2	12·3 ± 0·2	23·5 ± 0·2	< 0·005
3	6·7 ± 0·9	15·3 ± 1·5	< 0·05
4	4·7 ± 1·9	6·3 ± 0·1**	NS

Cells were grown for 3 days in low or normal calcium medium before cell cycle analysis. Results are means ± SD of triplicate cultures.

* Results for high and low calcium levels in individual experiments were compared using Student's unpaired *t*-test.

** Overall, high calcium significantly greater than low calcium; $P < 0·05$, paired *t*-test.

Cell morphology

Cells were examined by light and electron microscopy in low (one tenth normal) and normal calcium medium and in normal calcium medium in the presence of calmodulin antagonists. For electron microscopy, cultures were fixed in 3% glutaraldehyde and post-fixed in osmium tetroxide. After staining with uranyl acetate, samples were embedded in araldite and sections cut on a Reichert OMU3 ultramicrotome. Sections were mounted on grids and stained with lead citrate and examined in a Phillips EM 400 electron microscope.

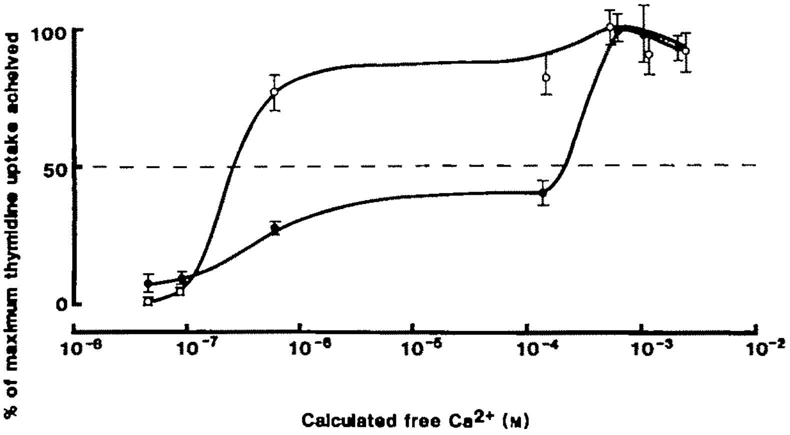

FIGURE 1. Effect of extracellular calcium on proliferation of normal human dermal fibroblasts (●) and neoplastic mouse B16 melanoma cells (○) in culture. Points are means ± SEM of triplicate cultures in a single representative experiment. The dotted line indicates the concentrations of extracellular calcium at which proliferation is reduced by 50%.

Extraction and measurement of intracellular calmodulin

Cells were washed thoroughly in PBS and scraped from the dishes, except for keratinocytes which were trypsinized to separate them from the collagen-coated dishes. All cells were suspended in a final volume of 1 ml of radioimmunoassay homogenization buffer (125 mM borate buffer with 1 mM EGTA and 75 mM sodium chloride, pH 8·4) and prepared for radioimmunoassay as previously described.[9] Supernatant calmodulin was related to total cell protein assayed by Lowry's method.[20] Incubations were performed in triplicate.

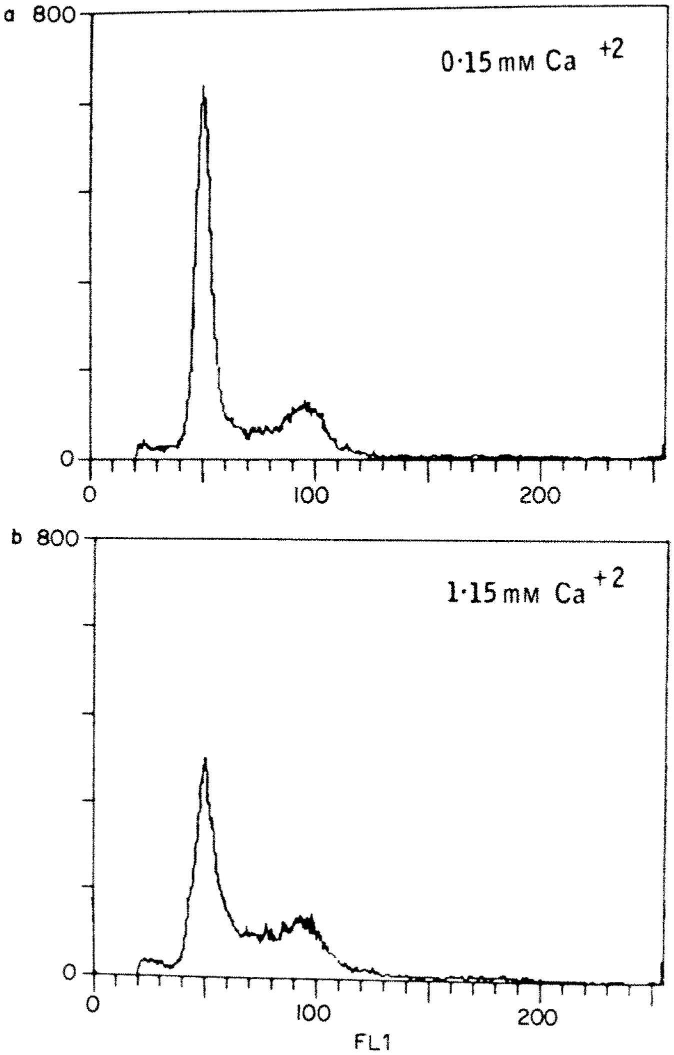

FIGURE 2. Effect of extracellular calcium on proliferation of normal human keratinocytes in culture. Cells were grown in (a) low (0·15 mM) and (b) normal (1·15 mM) calcium medium for 3 days before cell cycle analysis. Significantly more cells were in G1 (first peak) in (a) than in (b). The proportion of cells in G2 (second peak) was not significantly different, and, by subtraction, the proportion of cells in S phase was significantly lower in (a) than in (b). Results of an individual experiment are shown.

Statistical analysis

Student's paired an unpaired *t*-tests were used for statistical analysis.

RESULTS

The effect of extracellular calcium on cultured cell proliferation

Proliferation of all three cell types was decreased by reducing extracellular calcium, but B16 cells were less affected than were fibroblasts (Fig. 1 and Table 1) or keratinocytes (Table 2). In neoplastic B16 cells a 50% inhibition of proliferation was calculated to occur at a mean

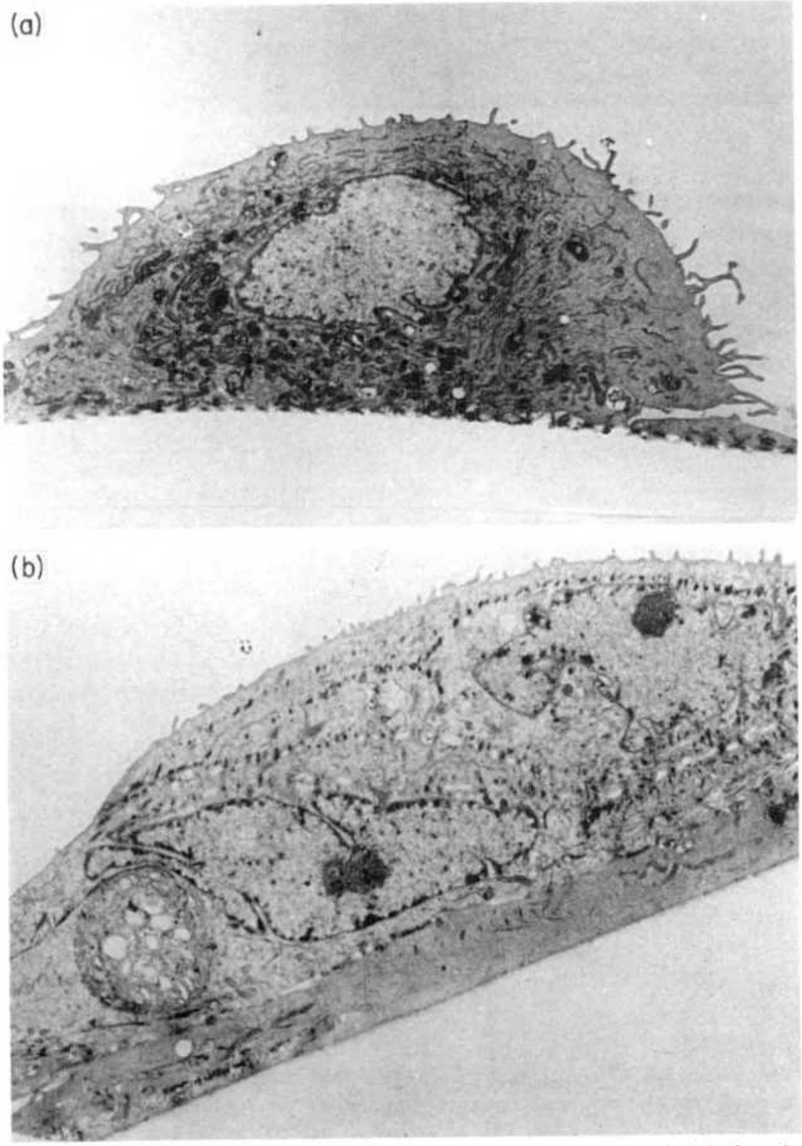

FIGURE 3. The morphology of keratinocytes grown in (a) low (0·15 mM) calcium (original × 3600) and (b) normal (1·15 mM) calcium (original × 1700).

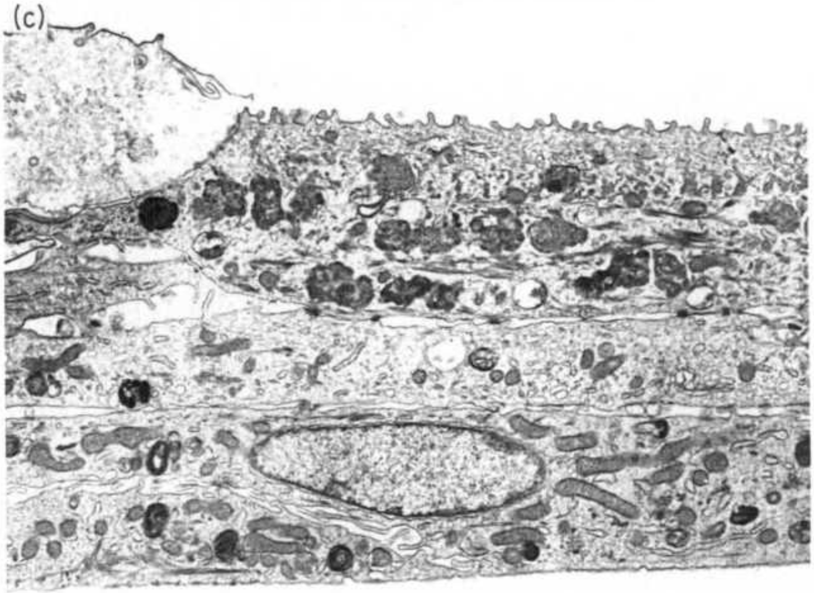

FIGURE 3 continued. The morphology of keratinocytes grown in (c) normal (1·15 mM) calcium with 12·5 μM iodo-C_8–W_7. (Original × 4600).

extracellular free calcium level (± SEM) of 0·25 ± 0·1 μM, whereas fibroblasts were 50% inhibited at 0·223 ± 0·07 mM calcium ($P < 0·001$). These values are based on a combination of [^{3}H] thymidine uptake and cell number indices of proliferation as summarized in Table 1.

For keratinocytes, lack of cells prevented examination of a full range of extracellular calcium concentrations as for other cells and proliferation was determined in normal (1·15 mM) and one tenth normal (0·15 mM) calcium only. Keratinocytes in the low calcium medium had a

TABLE 3. Effect of extracellular calcium on the intracellular concentration of calmodulin in cultured cells

		Calmodulin content (μg/mg cell protein, mean ± SD)		
Cell type	Experiment no.	Low calcium (0·15 mM)	Normal calcium (1·15 mM)	P*
Dermal fibroblasts	1	0·51 ± 0·10	0·50 ± 0·11	NS
	2	0·58 ± 0·06	0·58 ± 0·09	NS
B16 melanoma	1	2·90 ± 1·00	2·55 ± 0·25	NS
	2	0·88 ± 0·11	0·72 ± 0·30	NS
Keratinocytes	1	0·77 ± 0·10	1·50 ± 0·01	< 0·001
	2	0·37 ± 0·002	0·72 ± 0·12	< 0·05
	3	0·21 ± 0·03	0·74 ± 0·12	< 0·025
	4	0·78 ± 0·24	2·80 ± 0·64	< 0·025

* Student's unpaired *t*-test

significantly lower proportion of cells in S phase than cells in normal calcium medium in three out of four experiments ($P < 0{\cdot}05$) (Fig. 2 and Table 2). In our preliminary experiments, $[^3H]$ thymidine uptake did not reflect cell numbers for keratinocytes and was not used.

The morphology of keratinocytes in 0·15 mM calcium was clearly different to that of cells in normal calcium medium as illustrated in Figure 3. Cells in low calcium (Fig. 3a) grew as monolayers and contained abundant myelin bodies. Cells in normal calcium (Fig. 3b) were stratified and had numerous desmosomes.

The effect of extracellular calcium on intracellular calmodulin content

Intracellular calmodulin was determined in cells grown for 3 days in 0·15 mM or 1·15 mM calcium, (Table 3). For fibroblasts and B16 cells there was no significant difference in the calmodulin levels in low or normal calcium medium. For keratinocytes, however, there was significantly more calmodulin (2–3 times more) in cells in normal than in low calcium medium in each of three experiments despite considerable variation in the actual intracellular levels of calmodulin found between experiments.

There did not appear to be any clear difference between the intracellular levels of calmodulin present in the different cell types.

The effects of calmodulin antagonists on cell proliferation

The calmodulin antagonist W7 significantly inhibited the proliferation of all three cell types. Cells were grown for 3 days in Medium-199 (normal calcium) with concentrations of W7 between 1 and 100 μM. The potency of W7 against all three cell types was similar, as illustrated in Figure 4. The concentration of W7 required for 50% inhibition (IC_{50}) of proliferation was $16{\cdot}6 \pm 1{\cdot}2$ μM for dermal fibroblasts, $24{\cdot}5 \pm 1{\cdot}2$ μM for B16 melanoma cells and $20{\cdot}6 \pm 3{\cdot}0$ μM for human keratinocytes (mean ± SEM of two or three experiments as illustrated in Fig. 4).

W7 significantly increased the percentage of cells in G1 and reduced the percentage of cells in S phase. The percentage of cells in G2 was unaffected. This is illustrated in Figure 5. The analogue of W7, iodo-C_8-W7[21] was significantly more potent than W7 in inhibiting

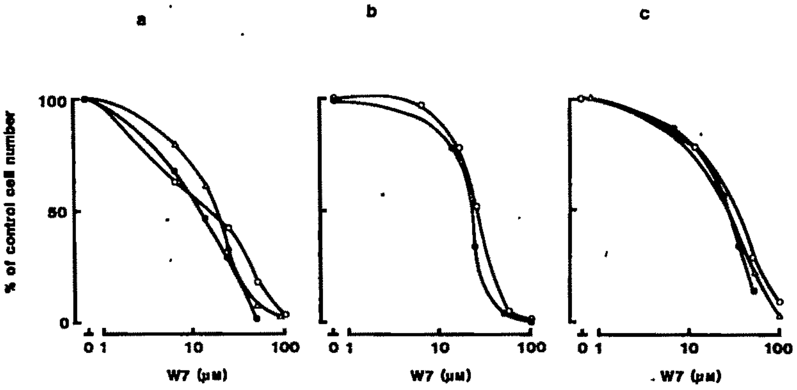

FIGURE 4. The effect of the calmodulin antagonist W7 on the rate of proliferation of (a) dermal fibroblasts, (b) B16 melanoma cells and (c) human keratinocytes. Cell number in the absence of drug was taken as 100% for the purposes of determining the concentration of drug producing 50% inhibition. Points are results of individual experiments.

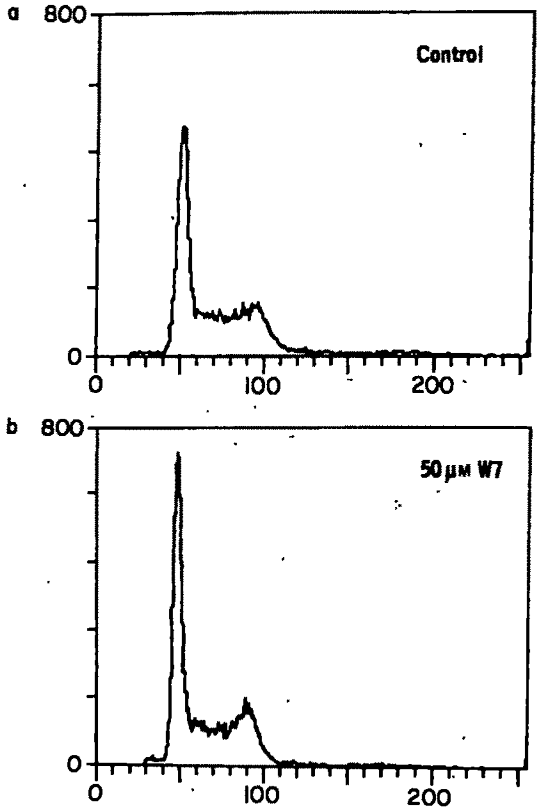

FIGURE 5. The effect of the calmodulin antagonist W7 on the cell cycle of normal human keratinocytes in culture. Cell cycle analysis of cells grown in (a) control medium and (b) medium containing 50 μM W7. W7 increased the percentage of cells in G1 (first peak), had no significant effect on the percentage of cells in G2 (second peak) and decreased the percentage of cells in S phase. Results of a single representative experiment are shown.

keratinocyte proliferation. The IC_{50} was $20{\cdot}6 \pm 3{\cdot}0$ μM for W7 and $10{\cdot}0 \pm 2{\cdot}0$ μM for iodo-C_8-W7, ($P < 0{\cdot}001$; unpaired *t*-test based on three experiments).

The morphology of keratinocytes cultured in iodo-C_8-W7 (12·5 μM for 3 days) is shown in Fig. 3c. The cells were stratified as would be expected of cells grown in normal calcium (see Fig. 3b), but they also contained myelin bodies, a feature common in cells grown in low calcium (see Fig. 3a).

DISCUSSION

Calmodulin levels are elevated 2–3 fold in the hyperproliferative lesional epidermis and $1\frac{1}{2}$ to 2 fold in non-lesional epidermis in psoriasis[1–4] and levels in lesional epidermis decrease with clearance of psoriasis.[6] Fairley *et al.*[2] reported an increase in mouse keratinocyte calmodulin associated with increased proliferation of these cells in culture. Such evidence strongly suggests that the increased calmodulin levels in the epidermis in psoriasis are related to the hyperproliferative state of the keratinocytes.

However, although calmodulin is involved in the regulation of DNA synthesis in normal and transformed cells, intracellular calmodulin levels are not necessarily elevated in transformed cells or rapidly growing cells.[8,9]

The objectives of the present study were, firstly, to examine the relationship between intracellular calmodulin and proliferation in human keratinocytes, comparing the 'behaviour' of the keratinocyte in this respect with that of normal dermal fibroblasts and neoplastic mouse B16 melanoma cells and, secondly, to examine the ability of calmodulin antagonists to inhibit the proliferation of keratinocytes.

The literature is divided on the response of keratinocytes to extracellular calcium. Hennings *et al.*[12] and Fairley *et al.*[2] reported that mouse keratinocytes grew faster in low (0·05–0·1 mM and 0·02 mM) calcium medium than in medium containing normal (1 to 1·5 mM) calcium. Tong *et al.*[14] and Hawley-Nelson *et al.*[13], however, found that mouse and human keratinocytes fail to proliferate in 0·09 mM and 0·2 mM calcium, respectively. These latter workers, interestingly, demonstrated that in 0·3 to 0·4 mM calcium cells grew faster than in 1·5 mM calcium. The differences do not appear to be due to species or even the criteria used for measuring cell proliferation. We, like others,[22] did not find [^{3}H] thymidine uptake reflected cell number of keratinocytes, and hence used cell cycle analysis and cell numbers for the present study. Other workers have used colony formation[13] or [^{3}H] thymidine uptake[12,14] as an index of cell proliferation.

In the current study, cells were initially plated in normal calcium medium and then transferred to low calcium medium as required. Other workers have plated cells directly into low calcium medium and reported hyperproliferation. It is possible that it is the initial concentration of extracellular calcium which dictates whether cells follow a differentiated, and possibly non-reversible, or proliferative programme. This has not been examined directly in our study, or in the other studies of keratinocyte proliferation, to the best of our knowledge.

In the present study, keratinocyte morphology in 0·15 mM calcium was clearly different to that in 1·15 mM, as found by other workers.[2,12,13] In low calcium, cells grew as monolayers with little sign of differentiation. In normal calcium, cells were stratified and more differentiated. Desmosome formation in keratinocytes is particularly dependent on extracellular calcium.[23]

Keratinocytes proved similar to normal fibroblasts in showing significantly reduced DNA synthesis in one tenth normal extracellular calcium in contrast to the response of neoplastic cells[11] which, like the B16 cells in the present study, proliferate until extracellular calcium is submicromolar.

Irrespective of the discrepancies in the literature on low calcium medium and keratinocyte growth, we were able to confirm the finding of Fairley *et al.*[2] that intracellular calmodulin levels appear to be related to the proliferative state of the keratinocyte in culture. This was not true for fibroblasts however. As B16 cells were unaffected by a 90% decrease in extracellular calcium we cannot say if calmodulin content is related to proliferation of these cells.

The relationship between intracellular calmodulin levels and the rate of cell proliferation is not clearly established. An attractive early hypothesis that the altered calcium sensitivity and abnormal growth characteristics of transformed cells might be attributable to increased levels of intracellular calmodulin[24] has not been substantiated by subsequent studies—many fast growing and transformed cells contain normal levels of calmodulin.[8,9] However, in psoriasis, calmodulin levels are significantly lower in lesions which have been successfully treated[6] suggesting that it is the level of calmodulin which may dictate the rate of proliferation of the epidermis.

Thus, the relationship between intracellular calmodulin content and proliferation displayed by human (and mouse[2]) keratinocytes in culture is more unusual than it at first seems. Some aspect of the calcium/calmodulin control of keratinocyte proliferation may differ significantly from that of other cells. We were not able to mimic *in vivo* the elevated calmodulin levels of lesional psoriatic epidermis by sellotape-stripping of normal epidermis,[1] although it is possible that this stimulus may simply not have been strong enough or applied for long enough.

Irrespective of whether the calcium/calmodulin control of keratinocytes differs in some aspects from that of other cells, calmodulin antagonists inhibited the proliferation of all three cell types equally. The ability of the antagonists to inhibit proliferation of keratinocytes in culture offers hope that psoriatic epidermis will be similarly responsive *in vivo*. Furthermore, our structure–activity studies[21] have established that both the potency and specificity of existing calmodulin antagonists can be significantly improved.

Our previous finding that anthralin has calmodulin antagonist properties[7] and the clinical improvement reported in response to topical application of the phenothiazine chlorpromazine[25] suggest that calmodulin antagonists should be considered seriously in the treatment of psoriasis. As we have shown that proliferation of normal keratinocytes in culture, like that of the psoriatic epidermis, seems linked to the level of calmodulin present, it is possible that the normal keratinocyte will provide an adequate *in vitro* model for the testing of such drugs.

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Methodology to measure the transient effect of occlusion on skin penetration and stratum corneum hydration *in vivo*

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SUMMARY

The effect of short duration occlusion on skin penetration and stratum corneum water content was studied *in vivo* in eight human subjects. Percutaneous absorption of hexyl nicotinate was monitored non-invasively by laser Doppler velocimetry (LDV) following each of three randomly assigned pre-treatments: untreated control, 30 min occlusion with a polypropylene chamber and 30 min occlusion followed by exposure to ambient conditions for 1 h. Stratum corneum water content after the same pre-treatments was measured with the dielectric probe technique. The local vasodilatory effect of the nicotinic acid ester was quantified using LDV by the onset of increased blood flow, the time of maximal increase in response, the magnitude of the peak response and the area under the response–time curve. Each of these parameters was significantly different, immediately following occlusion, from the untreated control values. However, if the occluded site was exposed for 1 h prior to hexyl nicotinate application these parameters did not differ significantly from the controls. Stratum corneum water content (expressed as a percentage of a maximal value) showed the same behaviour: the pre-treatment control value was 31·8 ± 4·8%; after 30 min occlusion, this had risen to 46·9 ± 6·2%; 1h later, the reading had returned to 32·1 ± 6·2%. There was a significant correlation between stratum corneum water content and area under the LDV response–time curve. It appears, therefore, that this method may be useful for quantifying the relationship between increased stratum corneum hydration and enhanced percutaneous absorption *in vivo* in man.

Occlusion of the skin and prevention of the insensible loss of body water causes hydration of the stratum corneum.[1] If the occlusive covering is impermeable to water molecules in the vapour phase, the water content of the stratum corneum beneath the occluding device will rise to a maximal value such that the chemical potential of water in this skin layer equals that in the lower aqueous regions of the tissue (i.e. a local equilibrium is established). It has been shown, *in vivo* in

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human subjects, that the increased hydration of the stratum corneum produced by occlusion can lead to an increase in the horny layer 'reservoir' and a promotion of penetrant percutaneous absorption.[2] The observation is considered, on the whole, a general one and is used practically in clinical dermatology to enhance, for example, the therapeutic effect of topically administered corticosteroids.

However, a quantitative relationship between level of hydration and skin penetration promotion has not been elucidated. In addition, the level of enhancement, at a particular degree of hydration, will almost certainly depend upon the physicochemical properties of the penetrant. This relationship, too, has not been quantified. We present here some experimental work in which we have attempted to design a methodology by which the link between skin hydration and promotion of percutaneous penetration can be determined *in vivo* in man.

METHODS

Eight healthy male volunteers (age range 22–35 years), with no history of skin disease, took part in the study. At the time of the experiment they were taking no prescribed medication. Informed consent was obtained in accordance with regulations overseen by the University of California, San Francisco, Committee on Human Research. Strenuous activity, hot drinks, non-prescription medications and alcohol were avoided for 12 h prior to the experimental procedures. For 15 min immediately before the study, the subjects rested quietly and acclimated to the ambient temperature (23 ± 2° C) and relative humidity (50–70%) of the environment in which the experiments were performed.

Measurements were made on the ventral forearm skin surface. Each individual observation required an area of approximately 2·5 cm^2. Experiments were conducted following one of three randomly assigned pre-treatments on different skin areas: untreated control (30 min exposure of the skin to ambient conditions), occlusion for 30 min using a small polypropylene (Hilltop®) chamber, and 30 min occlusion followed by exposure to ambient conditions for 1 h. Assessment of the effects of these treatments on percutaneous absorption and skin hydration were made using laser Doppler velocimetry[3] and the dielectric probe technique,[4] respectively. It should be emphasized that each of the eight subjects underwent each of the three treatment protocols using both investigative techniques. An individual subject provided, therefore, his own control and permitted, as a result, paired comparisons to be made.

Laser Doppler velocimetry

The laser Doppler velocimetry (LDV) technique has been previously described.[3] Following pre-treatment, 10 mM hexyl nicotinate in a 60:40 v/v mixture of propylene glycol and isopropanol was applied topically on a saturated filter paper disc for 15 min. The disc was covered to prevent loss of isopropanol by evaporation. At the end of the application period, the patch was removed, excess solution was wiped away with tissue, and the LDV (Medpacific LD5000) probe, was affixed to the skin in the centre of the pre-treated area with a double-sided adhesive disc. The time-course of the resultant nicotinate-induced vasodilatation was monitored over the next 90 min as previously described.[5] All LDV measurements were corrected for pre-treatment basal flow readings and for occasional small instrumental baseline drift ($\leqslant$30 mV). The pharmacological response was characterized by the time of onset of action (the time at which a definite and sustained increase in LDV output occurred), the time to peak response (defined as the time after the onset of action, at which d(LDV output)/dt was < 5mV/min), the magnitude of the peak response, and the area under the response–time curve. While there is no a priori reason for analysis to be performed on each of these four parameters, our present database does not unequivocally show which measurement is most sensitive. Therefore, all characteristics of the response–time curve were evaluated for significance. Statistical analysis of the data employed analysis of variance and paired *t*-tests, and linear regression.

Dielectric probe technique

The microwave probe approach permits dielectric measurements of stratum corneum water content to be made non-invasively *in vivo*.[4,6] The microwave frequency (1 GHz) was determined by the requirement that the radiation interact directly with water molecules while minimizing interactions with salts and proteins. The probe reacts, therefore, to the number of water molecules present and detects their small degree of rotation in response to the applied microwave field. The probe response has been shown to be a linear function of water content.[4,6] Design of the probe is important because microwaves can penetrate several centimetres into biological tissue. Therefore, the probe used in the present work consisted of many interdigitated fingers of gold bonded to a glass substrate. The gaps separating the fingers were 10 μm. This configuration confines the microwave field to within a depth of a few microns and it can be used, in conjunction with inert spacers of different thickness, to measure the water concentration profile across the stratum corneum *in vivo*.[4,6] The probe was calibrated by measuring its response to a drop of pure water and to air. The deflection recorded from water (2·15 V) corresponds to a relative water content of 100%. No deflection is recorded from air and this, therefore, represents the zero baseline. Measurements for skin are then expressed as relative water content between these two extremes. After calibration, relative stratum corneum water contents were evaluated following the three pre-treatments described above. Measurements involved taking dielectric probe readings in direct contact with the skin surface and through a 6 μm thick Teflon sheet. The difference between the probe outputs from these two measurements yielded a voltage deflection which was then converted to a percentage relative water content. The Teflon spacer significantly attenuates the signal picked up by the probe. Measurements were made in this way to minimize day-to-day fluctuations in probe sensitivity and other environmental factors.

RESULTS

The LDV results after topical application of hexyl nicotinate following the three pre-treatments are shown in Figure 1. Time of onset of action, time to peak response, magnitude of peak response and area under the response–time curve are listed in Table 1 which also shows the relative stratum corneum water content levels determined by the dielectric probe technique at the end of the pre-treatment periods.

Statistical analysis showed that a 30-min period of occlusion significantly shortened both the time of onset of the LDV-detected response to hexyl nicotinate and the time to peak response when compared to the untreated controls ($P < 0{\cdot}05$). In addition, the magnitude of the peak LDV response to hexyl nicotinate and the area under the response–time curve were significantly increased ($P < 0{\cdot}05$). If the occluded site was exposed to ambient conditions for 1 h after occlusion before nicotinate application, blood flow measurements were not significantly different from the control values.

The stratum corneum water content values showed the same pattern. Exposure for 1 h after occlusion gave results indistinguishable from the controls, whereas the horny layer water content after 30 min occlusion was significantly elevated ($P < 0{\cdot}001$). There was a significant

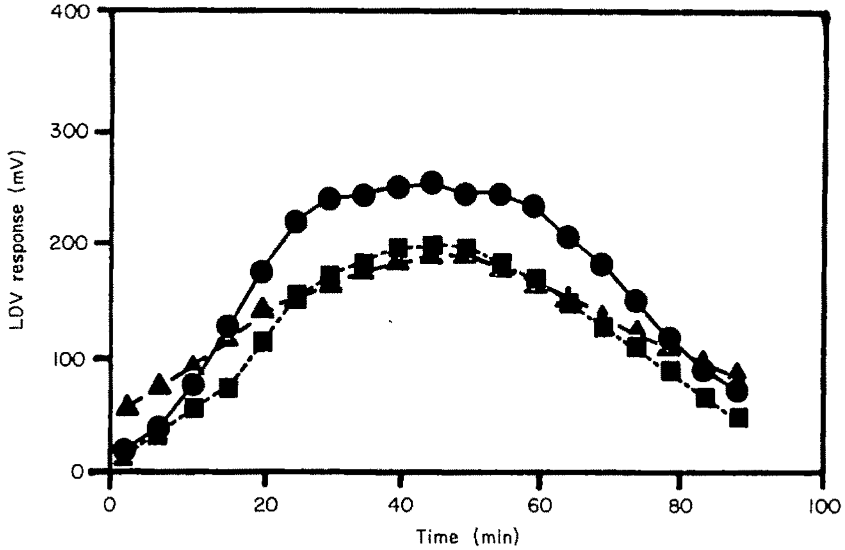

FIGURE 1. LDV responses (expressed as change from baseline) to hexyl nicotinate as a function of time following three pre-treatments of the drug application site: untreated control (■), 30-min occlusion (●), and 30-min occlusion followed by 1 h exposure to ambient conditions (▲). Points are means for eight subjects; the SEM for each point was within the range of 20–40 mV and most were < 30 mV.

correlation between stratum corneum water content and area under the LDV response–time curve after 30 min occlusion ($r = 0{\cdot}8$) ($P < 0{\cdot}05$).

It should be noted that the data obtained after occlusion followed by 1 h exposure generally gave the largest degree of variability. This probably reflects inter-subject variation in skin dehydration rates following a period of occlusion. Extending the period of ambient exposure following occlusion might reduce this variation.

TABLE 1. Effects of pre-treatment on LDV-assessed responses to topical hexyl nicotinate, and dielectric probe measurements of stratum corneum water content values (values are means ± SEM; $n = 8$)

Pre-treatment	Time of onset of action (min)	Time to peak response (min)	Magnitude of peak of response (mV)	AUC* (mV × h)	Stratum corneum water content† (%)
None	12·5 ± 4·0	35·0 ± 3·8	210 ± 28	198 ± 34	31·8 ± 4·8
30-min occlusion	5·6 ± 2·6‡	29·4 ± 2·6‡	254 ± 25‡	267 ± 35‡	46·9 ± 6·2§
30 min occlusion + 1 h ambient exposure	10·6 ± 3·0	37·8 ± 18·5	242 ± 78	213 ± 71	32·1 ± 6·2

* AUC = area under the LDV-detected response–time curve.

† Values expressed as a percentage of the probe response to a drop of pure water.

‡ Significantly different from control ($P < 0{\cdot}05$).

§ Significantly different from control ($P < 0{\cdot}001$).

DISCUSSION

The preliminary results presented here are consistent with the generally accepted role of skin hydration or occlusion on percutaneous absorption, namely, that penetration is greater through hydrated skin. Effects of occlusion on drug delivery across the skin have been studied in detail and extensively reviewed.[1,2,7] Much *in vitro* work has been, and continues to be, conducted with fully hydrated skin tissue.[8] The absorption-enhancing effects of occlusion are employed to good purpose in clinical dermatology.[1,2]

In vivo, however, while the qualitative action of occlusion is clear, the magnitude and duration of the effects induced are less well characterized. The current investigation serves to offer a methodological approach to the better understanding of skin hydration *in vivo*. The combination of non-invasive techniques permits evaluation of both percutaneous penetration and stratum corneum water content and determination of their functional dependence upon each other. Although the LDV test employed here generates a response which is due to a combination of both skin absorption and microvascular stimulation, extensive previous work[9] has shown that the approach is valid when nicotinate levels applied correspond to the linear portion of the dose–response curve. Clearly, however, two levels of biological variability are built into this experiment and these constitute a limitation.

A logical extension of the work would be to include transepidermal water loss measurements as a further non-invasive procedure to evaluate barrier function status. Obviously, as with our recent use of LDV to explore the action of penetration enhancers *in vivo*,[5] there are a number of experimental variables which now need to be studied systematically to investigate the details of the occlusion time–penetration enhancement–water content equation. The simplicity of the experimental system and the ease with which measurements can be made *in vivo* in humans warrant the performance of further investigations.

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The effect of oral retinoid therapy on the normal human immune system

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SUMMARY

Twenty four patients were studied prior to and after 6 and 12 weeks therapy with isotretinoin (17 patients) for acne and related disorders, or with etretinate (7 patients) for psoriasis and related disorders. Patients treated with isotretinoin had a significant reduction in natural killer cell activity at an effector: target cell ratio of 100:1 at 12 weeks and also a reduction in natural killer cell numbers at this time. Patients treated with etretinate had elevated natural killer cell activity and a significant elevation of natural killer cell numbers at 12 weeks. Other tests which were performed and showed no significant change at 6 or 12 weeks compared with starting levels included lymphocyte transformation in response to phytohaemagglutinin, pokeweed mitogen and concanavalin A, total numbers of circulating T lymphocytes, B lymphocytes and T helper and T suppressor subsets, numbers of epidermal Langerhans cells and serum levels of IgA, IgM and IgE. In view of the involvement of natural killer cells in the initial phase of organ rejection, these results suggest that isotretinoin is the safer of the two retinoids if administration to renal transplant recipients is considered, particularly in the immediate post-transplant period.

In the past decade the introduction of oral retinoid therapy has been a major step forward in dermatological therapeutics. The two retinoids currently prescribable in both Europe and North America are isotretinoin (Roaccutane® U.K., Accutane® U.S.A.) and etretinate (Tigason® U.K., Tegison® U.S.A.). Isotretinoin is of great value in control of severe nodulocystic acne,[1,2] and etretinate is an advance in our management of the dyskeratoses, in particular psoriasis.[3,4] A third major potential area for clinical use of oral retinoids is in the treatment and prevention of premalignant and malignant skin disease. Following encouraging early reports in the mouse papilloma model,[5] a number of clinical studies have reported encouraging results in actinic keratoses,[6,7] basal cell carcinoma,[8,9] and squamous cell carcinoma.[10] From these reports it would appear that relatively high doses of retinoid are required to have an effect, and that continuous treatment is required to prevent relapse.

It is now well established by the work of several groups that a long term complication of renal

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transplantation and the necessary associated therapeutic immunosuppression is the development of large numbers of both premalignant and also malignant skin tumours, mainly on light exposed skin.[11-13] The numbers of lesions appear to increase with time after transplantation. Although one study has suggested that a metabolite of azathioprine might be responsible,[14] there appears at present to be no reduction in the rate of development of cutaneous lesions associated with the current switch in many transplant centres from azathioprine to cyclosporin. Animal work suggests that cutaneous malignancy is likely to remain a problem in patients given cyclosporin, with no prior azathioprine,[15] and clearly, as technical problems of organ transplantation are overcome, and a cohort of long-term survivors is established, the cutaneous problems are likely to assume greater importance. Oral retinoid therapy might be of great value in this cohort of patients, provided that it was firmly established that retinoid treatment did not increase the risk of rejection of the transplanted organ.

The current literature on the immunological effects of retinoid therapy is confused, with varying results reported for animal models and man. In general the studies in man are confined to small studies of patients looking at only one test of immune function.[16-18]

Against this background, we have carried out a battery of tests of immune function, studying patients prior to and during therapy with both etretinate and isotretinoin. We felt it essential that this data was available before proceeding to consider oral retinoid therapy in organ transplant recipients, so that the choice between the two currently available retinoids could be made on logical grounds.

METHODS

Patients

Twenty four patients were studied in all. Seventeen patients (12 males, five females, mean age 26 years, range 18–49 years) received isotretinoin 1 mg/kg/day for 12 weeks. Fourteen of this group were treated for severe acne vulgaris non-responsive to oral antibiotics, two for rosacea, and one for hidradenitis suppurativa.

Seven patients (three males, four females, mean age 57 years, range 44–76 years) were treated with etretinate 0·5–0·75 mg/kg daily. Six of this group were treated for severe psoriasis vulgaris resistant to topical therapy, and one for ichthyosis vulgaris. Ethical Committee approval was obtained for the study.

Immunological methods

The immunological tests carried out included the following: Serum levels of circulating IgG, IgA and IgM were measured by standard radial immunodiffusion, and of IgE by a radioallergosorbent technique. Lymphocyte responses were measured to two concentrations of the mitogens phytohaemagglutinin (PHA) (0·25 μg and 0·1 μg PHA/10^6 lymphocytes) pokeweed mitogen (1:10 and 1:100 dilutions of a 1 mg/ml solution) and concanavalin A, (0·25 μg and 0·025 μg/10^6 lymphocytes). For the mitogen assays a concentration of 10^6 purified lymphocytes/ml was assayed in triplicate; 256 μl autologous plasma was added to each well, and control wells contained only 25 μl of RPMI containing L-glutamine 2 mmol/ml, penicillin/streptomycin mixture 2 U/ml, and 5% heat inactivated foetal calf serum (all from Gibco, Paisley, U.K.). After 72 h culture at 37°C in 5% CO_2, plates were pulsed with 25 μl ^{3}H thymidine per well (1 μCi per well) and further incubated overnight. Thymidine was omitted from one control well to provide the background level. After 18 h exposure to tritiated thymidine, the plates were harvested on a Skatron cell harvester, and samples counted in a

Tricarb series 3 scintillation counter (Packard). Results were calculated with the mean taken of each of the triplicate assays, and the mean of controls without tritiated thymidine subtracted. Results are expressed as counts per 10^6 cells.

Langerhans cells were quantitated by the standard suction blister technique. A 2 cm diameter area of normal epidermis was obtained by raising a blister on the upper inner arm by applying a 2 cm diameter suction cup and negative pressure for 45 min. The epidermis was then removed, snap frozen and stored at −20°C. All epidermal sheets were examined within 20 days of removal. Once thawed, the epidermal sheet was stained using a standard indirect immunoperoxidase technique.[19] Anti-HTA1 (Sera Laboratories) was used as a marker for Langerhans cells at a 1:100 dilution in Tris buffer. After visualization of Langerhans cells with 0·03% diamino benzidine, sections were permanently mounted and Langerhans cells counted per square millimetre. Langerhans cell bodies were only counted if a minimum of three dendrites were clearly identified emerging from the body of the cell.

For quantitation of T cell subsets, B cells and NK cells, all patients were venesected between 0830 and 0930 h to minimize effects due to diurnal variation. Heparinized blood samples were diluted in Hanks balanced salt soluion without calcium and magnesium. Mononuclear cells were separated on a Ficoll-Hypaque (lymphoprep) gradient and the cells washed twice for 10 min in phosphate buffered saline. Twenty microlitres of antibody was added to 200 μl of phosphate buffered saline (PBS) containing 1×10^6 mononuclear cells, and incubated on ice for 30 min. The antibodies used were CD3 pan T marker UCHT-1 (Seward, Bedford, U.K.), CD4 Dako T4 helper marker (Dako, U.K.), CD8 T suppressor/cytotoxic marker (Scottish Antibody Production Unit, Law Hospital, Carluke, Scotland) B cell marker B1 (Coulter) and natural killer cell marker Leu 7 (Becton Dickinson). After two further washes in PBS, cells were incubated for a further 30 min on ice with 100 μl of a 1:10 dilution of rabbit anti-mouse immunoglobulin F $(ab)^2$ fragment (Dako) in PBS, conjugated to fluorescein isothyocyanate. After two further washes, cells were counted using a FACS analyser (Becton Dickinson Immuno Cytometry Systems, Mountain View, CA, U.S.A.). Correlation of wide angle light scatter and cell volume enabled lymphocytes to be resolved from monocytes, and also from any contaminating granulocytes. Gates were placed around the lymphocyte population to exclude all other cells, fluorescence signals from 1×10^6 lymphocytes were analysed for each antibody.

For natural killer cell function, the mononuclear cell fraction of heparinized blood was isolated as detailed above. The lymphocytes were then washed three times in RPMI containing L-glutamine 2 mmol/ml, 2 U/ml of penicillin/streptomycin mixture and 5% heat inactivated foetal calf serum (Gibco). Cells were then resuspended in a small volume of this medium and counted. Viability was assessed by trypan blue exclusion and was never less than 95%. A final cell concentration of 2×10^6/ml was achieved and two further dilutions made for concentrations of 1×10^6/ml and 5×10^5/ml.

Target cells used in this assay were the erythroleukemic cell line K562, maintained in continuous culture in RPMI with additions as detailed above. Cells were sub-cultured routinely every 2 days and always 24 h before performing a cytotoxicity assay. An aliquot of culture medium containing 3×10^6 cells was incubated with 0·2 ml of sodium chromate^{51}, containing 20 MBq/ml activity, at 37°C for 1 h. The cells were then washed six times in RPMI plus additions and the final concentration adjusted to 2×10^4/ml. For the cytotoxicity assay, V bottomed micro titre plates (Flow) were used; 0·1 ml of each of the effector cell concentrations (2×10^6, 1×10^6 and 5×10^5) were incubated with 0·1 ml of the target cell preparation to achieve effector to target cell ratios of 100:1, 50:1 and 25:1. Effector cells and labelled target cells were incubated together for 4 h at 37°C in 5% CO_2. Two controls were used, the first to estimate the degree of spontaneous release of isotope from 0·1 ml of labelled target cells that had been incubated with 0·1 ml of medium alone, and the second to estimate the total activity by completely lysing target cells by the addition of 0·1 ml of 5 M sodium hydroxide to wells containing 0·1 ml of labelled target cells. All tests and controls were carried out in quadruplicate, and, following incubation, 0·1 ml of the cell free supernatant was removed from each well and counted for 3 min in a gamma counter (LKB 1282 Compugamma) to assess radioisotope release. Results were expressed as percentage lysis according to the following formula:

$$\text{Percentage lysis} = \frac{\text{mean CPM sample} - \text{mean CPM spontaneous release}}{\text{mean CPM total activity} - \text{mean CPM spontaneous release}} \times 100$$

where CPM is counts per minute.

All changes at 6 and 12 weeks compared with baseline levels were investigated for levels of significant variation by means of the paired Student's *t*-test. Statistical significance was calculated at the 5% level.

RESULTS

Isotretinoin treated patients

In this group of patients, natural killer cell activity declined at all effector: target ratios at 6 and

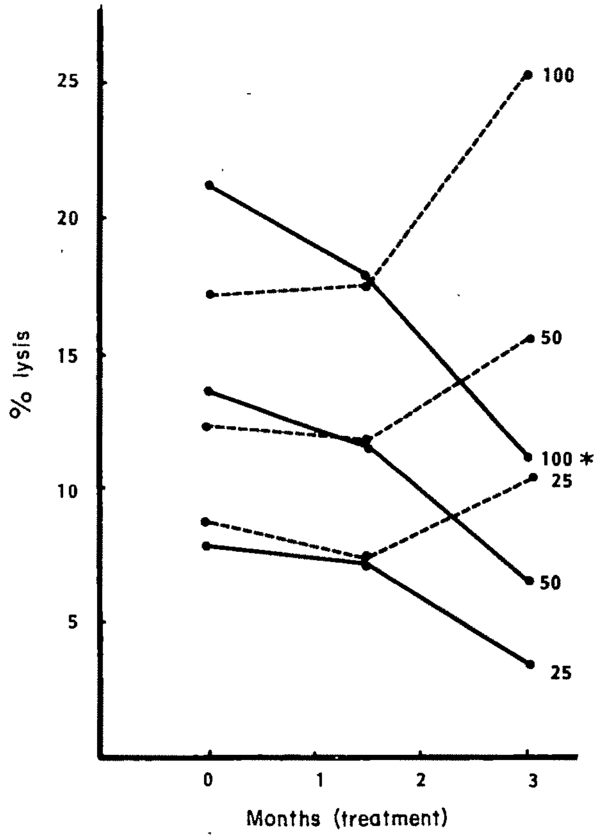

FIGURE 1. Natural killer cell function during therapy with oral etretinate (-----) and isotretinoin (———) measured at effector: target cell ratios of 100:1, 50:1 and 25:1. $^{*}P<0{\cdot}05$ compared with baseline.

TABLE 1. Natural killer (NK) cell numbers during etretinate and isotretinoin therapy

	NK cell numbers/10^4 lymphocytes		
	Prior to therapy	Week 6	Week 12
Isotretinoin	551	516	470
Etretinate	636	628	809*

* $P < 0{\cdot}05$ compared with baseline.

12 weeks and a significant reduction was seen at 12 weeks with an effector: target ratio of 100:1 (Fig. 1). Numbers of circulating natural killer cells fell during this period, but this change did not achieve statistical significance (Table 1).

A statistically significant rise in levels of circulating serum IgG was seen at 12 weeks by comparison with pre-treatment levels, but levels at 0, 6 and 12 weeks are all within the normal range for our laboratory. There were no significant changes in any of the other tests performed (Table 2).

If the two patients with rosacea and one with hidradenitis are excluded from analysis, the 14 patients with acne vulgaris also showed a significant decline in natural killer cell activity and an elevation of serum IgG.

TABLE 2. Lymphocyte numbers, mitogen responses, total immunoglobulins and Langerhans cell numbers in seven patients prior to and after 6 and 12 weeks of isotretinoin therapy

			Week no.		
		Normal range	0	6	12
Circulating lymphocyte numbers/10^4 mononuclear cells	Total T lymphocytes	650–1900	1825 (631)	1697 (634)	1825 (663)
	Helper T cells	470–1300	1122 (542)	1095 (409)	1135 (438)
	Cytotoxic suppressor T cells	180–750	820 (388)	712 (274)	770 (333)
	B lymphocytes		251 (128)	267 (150)	340 (228)
Circulating immunoglobulin levels	IgG mg/100 ml	700–1900	1086 (228)	1109 (235)	1264 (221)*
	IgA mg/100 ml	90–450	188 (64)	197 (62)	207 (61)
	IgM mg/100 ml	45–180	106 (37)	109 (39)	116 (32)
	IgE IU/ml	0–100	107 (162)	124 (228)	96 (76)
Mitogen stimulation indices	PWM (1:10)		57·2	79·3	102·1
	PWM (1:100)		69·5	82·6	99·1
	Con A (1:10)		77·7	86·2	107·0
	Con A (1:100)		14·4	15·7	99·1
	PHA (1:100)		261·4	387	573
	PHA (1:250)		200·4	267·3	398
Langerhans cells/mm^2		882 (112)	939 (212)	ND	998 (188)

* $P = 0{\cdot}001$ compared with week 0.
ND = not determined.
Values in parenthesis are standard deviations.

TABLE 3. Lymphocyte numbers, total immunoglobulins, mitogen responses and Langerhans cell numbers in 17 patients prior to and after 6 and 12 weeks of etretinate therapy

			Week no.		
		Normal range	0	6	12
Circulating lymphocyte numbers/10^4 mononuclear cells	Total T lymphocytes	650–1900	1599 (231)	1585 (312)	2008 (228)
	Helper T cells	470–1300	1012 (412)	938 (314)	1301 (402)
	Cytotoxic suppressor T cells	180–750	665 (353)	725 (396)	886 (380)
	B lymphocytes		303 (176)	370 (290)	372 (268)
Circulating immunoglobulin levels	IgG mg/100 ml	700–1900	1009 (221)	1025 (208)	1068 (141)
	IgA mg/100 ml	90–450	264 (113)	263 (100)	264 (94)
	IgM mg/100 ml	45–180	108 (29)	118 (37)	99 (39)
	IgE IU/ml	0–100	111 (138)	167 (118)	100 (120)
Mitogen stimulation indices	PWM (1:10)		56·4	36·5	70·3
	PWM (1:100)		44·2	33·7	67·3
	Con A (1:10)		41·4	32·1	65·8
	Con A (1:100)		10·4	6·9	9·5
	PHA (1:100)		89·3	161·6	229·0
	PHA (1:250)		91·9	108·6	167·4
Langerhans cells/mm^2		882 (112)	812 (212)	ND	752 (193)

ND = not determined.
Values in parentheses are standard deviations.

Etretinate treated patients

In this group of patients, natural killer cell numbers were significantly raised at 12 weeks and natural killer cell activity was also raised at 12 weeks, although this elevation did not reach statistical significance by comparison with pre-treatment levels. No other significant changes in tests of immune function were observed in the etretinate treated group (Table 3). Analysis of the six patients with psoriasis, with the exclusion of the one patient with ichthyosis, also revealed a significant elevation of natural killer cell activity at 12 weeks and a significant elevation in natural killer cell numbers at both 6 and 12 weeks.

DISCUSSION

The finding of particular interest in this study is the fact that natural killer cell numbers and function alter over a 12-week treatment period with both isotretinoin and etretinate, but in opposite directions. With isotretinoin the NK cell numbers and function are reduced, while with etretinate they are increased. The observed elevation of serum IgG levels in the isotretinoin treated group confirms an earlier study,[16] but is unlikely to be of biological significance in view of the fact that the highest levels reached were still within the normal range of our laboratory.

A previous study carried out by Jansen *et al.*[20] studied six patients with psoriasis, one patient with verruca vulgaris, one patient with condylomata accuminata and one patient with acne vulgaris. Seven patients received etretinate therapy in doses of 25–75 mg daily and two received isotretinoin in daily doses of 10–20 mg. The authors report an 'increasing trend' for NK activity during the first two months of treatment. Pigatto *et al.*[21] assessed natural killer cell function in eight patients with psoriasis who received etretinate and nine patients with acne who received isotretinoin only, 2 weeks after starting therapy. They report increased NK function in the psoriatic etretinate treated group and no change in the acne isotretinoin treated group at this point in time. Kang and Ellis[22] report no change in natural killer cell activity in patients treated with etretinate, 28 days after starting therapy.

All of these studies and our own, clearly make the basic assumption that the diseases for which retinoids are given are not associated with any general underlying immune defect which might alter either the initial findings or the observed changes during retinoid therapy. The fact that pre-treatment levels in all tests used in our study were within the normal range supports this assumption. There were no significant differences in pre-treatment results between our etretinate and isotretinoin treated groups, which do of course differ in their age range because of the use of isotretinoin in young acne patients. The relatively small number of patients in the etretinate treated group reflects the number of patients in this centre commencing etretinate over the 9-month period of this study, and continuing therapy for at least 12 weeks.

Our results show that it is important to report the effects of isotretinoin and etretinate on the immune system separately. It is not acceptable to group together results obtained in patients treated with these two retinoids. Their effects on the pilosebaceous follicle in acne are also very different and this difference clearly extends to their effect on natural killer cells. The possibility that natural killer cells are involved in graft rejection has been discussed in the past. Mowatt and Felstein[23] investigated natural killer cell activity in murine graft-versus-host disease and suggest that natural killer cells may well be a non-specific component of immunological tissue damage. Similarly, Nemlander, Saksela and Hayry[24] have identified natural killer cells in rat renal allografts during rejection and have also found natural killer cell activity to be high in the early rejection phase. Roy *et al.*[25] have also reported increased natural killer cell activity in the early phase of the graft-versus-host reaction, and Dokhelar *et al.*[26] demonstrate increased natural killer cell activity 9 days after receipt of bone marrow transplantation and also report a statistically significant correlation between high numbers of natural killer cells and acute graft-versus-host disease in the 1st month after receipt of a transplant. All these studies suggest that natural killer cells may well be involved in the early phases of graft rejection and graft-versus-host disease.

These data, together with the results of our study, suggest that if oral retinoids are to be used in renal transplant patients, particularly in the immediate post-transplant period, isotretinoin is less likely than etretinate to be associated with any increased risk of organ rejection. The work carried out to date on the relative effects of isotretinoin and etretinate in reversing or preventing premalignant cutaneous lesions suggests that both are effective in this situation.[27] It should, however, be emphasised that, to date, these results are associated with long term continuous use of retinoid. The fact that at 12 weeks the trend for both NK cell numbers and activity shows a steep upward curve for etretinate is a further point to suggest that isotretinoin is likely to be the safer drug over a longer period. A further note of caution should be added with regard to etretinate in view of the recent case reports of patients developing malignancies during or shortly after etretinate therapy.[28,29] No such reports are in print for isotretinoin.

Further work on NK cell numbers and function in patients treated for longer than 3 months with etretinate is in progress.

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Aminoterminal propeptide of type III procollagen in methotrexate-induced liver fibrosis and cirrhosis

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SUMMARY

Twenty-four psoriatic patients on methotrexate were studied with liver biopsies and serum measurements of aminoterminal propeptide of type III procollagen (PIII NP). All but one of nine patients with serum levels of PIII NP above the normal range had liver fibrosis or cirrhosis and no normal liver biopsies were obtained in this group. In contrast, nine normal liver biopsies and two biopsies with minimal fibrosis were found among the 15 patients with normal serum levels of PIII NP. The study indicates that aminoterminal propeptide of type III procollagen can be utilized as a valuable non-invasive marker of fibrogenesis in the liver. This analysis is not specific for the liver, but it seems that the number of liver biopsies probably can be reduced in psoriatics on methotrexate who have normal levels of PIII NP.

It is well established that methotrexate (MTX) may induce liver damage, which in some MTX-treated psoriatics will lead to fibrosis and cirrhosis.[1,2] Liver biopsies are the most reliable method of assessing MTX-induced liver damage, and liver biopsies are recommended in the monitoring of MTX-treated psoriatics.[3] Obtaining percutaneous specimens is, however, an uncomfortable procedure with a certain morbidity.[4] Several attempts have, therefore, been made to test non-invasively,[5–9] but none of these tests, including serum analyses, have been sufficiently successful to be substituted for histology. It is also unlikely that single laboratory tests can make biopsies unnecessary. However, with relevant serum measurements it may be possible to judge when a liver biopsy is indicated. Recently, serum concentrations of the aminoterminal propeptide of type III procollagen (PIII NP) have been measured as a means of clinical chemical diagnosis and follow-up of fibrotic liver diseases.[10,11] We report here PIII NP

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levels determined by a new rapid equilibrium type of radioimmunoassay in relation to histology of liver biopsies in MTX-treated psoriatics. This new method[12] which is based on the human propeptide is more suitable for routine clinical chemical use than earlier methods using a bovine propeptide.

METHODS

Twenty-four psoriatics, 14 males and 10 females, aged 32 to 75 years (median age 50 years) treated with MTX for at least 6 months (mean 7 years) were studied. All patients were on a weekly divided oral dosage, in general receiving three doses of 5 mg MTX with 12 h intervals once weekly. Eight patients had previously been treated with etretinate. Three had received arsenic and vitamin A, and three prior to MTX-treatment had had a high daily consumption of alcohol (Tables 1 and 2).

All patients had liver biopsies taken during MTX-treatment at intervals of 1 to 2 years. In all patients the histological specimens were obtained by the Menghini technique. All biopsies were stained with haematoxylin and eosin, periodic acid-Schiff reagent, van Gieson's stain, Masson's trichrome stain, with Pearl's method, and reticulin fibre stain The diagnosis of fibrosis or cirrhosis was made by the pathologist (H.S.) without knowledge of the clinical or laboratory data. In none of the patients had initial liver biopsies, taken prior to or early on in treatment, shown cirrhosis or more than slight fibrosis. The biopsy taken closest to the time of measurement of PIII NP was used in the present study.

Serum for PIII NP measurement was obtained from each patient and stored at −20°C. Serial measurements of PIII NP were performed on one patient, a 41-year-old man suffering from pustular psoriasis and psoriatic arthritis who was treated periodically with 15 mg MTX weekly (Fig. 1). MTX had been discontinued first owing to greatly elevated serum transaminase levels, and PIII NP was measured shortly after this. It should be noted that the patient had consumed

TABLE 1. Methotrexate-treated patients with elevated serum levels of aminoterminal propeptide of type III procollagen (>4·2 μg/l)

Patient no.	Sex	Age (years)	Diagnosis	Liver histology	Duration of MTX (years)	Other hepatotoxic factors	PIII NP (μg/l)
1	M	71	P+A	Pronounced progression in steatosis	6	—	7·6
2	F	64	PP	Fibrosis	3	Tigason	7·4
3	F	73	P	Fibrosis	7	Arsenic Vitamin A	7·2
4	M	37	P+A	Fibrosis	11	—	5·9
5	M	61	P+A	Cirrhosis	11	Alcohol	
6	F	36	P+A	Fibrosis, previously cirrhosis	16	—	4·8
7	F	56	P+A	Fibrosis	11	Arsenic Vitamin A	4·6
8	M	70	P	Fibrosis	9	Alcohol	4·5
9	F	53	P+A	Cirrhosis	20	—	4·3

P = psoriasis vulgaris, PP = pustular psoriasis, A = psoriatic arthritis.

TABLE 2. Methotrexate-treated patients with normal serum levels of aminoterminal propeptide of type III procollagen (1·7–4·2 μg/l).

Patient no.	Sex	Age (years)	Diagnosis	Liver histology	Duration of MTX (years)	Other hepatotoxic factors	PIII NP (μg/l)
10	M	65	P	Fibrosis	4	Tigason	4·1
11	M	40	PP	Fibrosis	4	—	4·1
12	F	56	P+A	Normal	9	—	3·9
13	F	41	P	Slight fibrosis	10	—	3·7
14	M	60	PP	Slight fibrosis	½	Tigason	3·5
15	M	74	P	Normal	10	Alcohol Tigason	3·5
16	F	61	P	Cirrhosis	6	Tigason	3·4
17	M	35	P+A	Normal	7	—	3·4
18	M	40	P	Normal	6	Tigason	3·4
19	M	75	P	Fibrosis, previously cirrhosis	16	Arsenic Vitamin A	3·1
20	F	32	P+A	Normal	2	—	3·1
21	M	36	P+A	Normal	4	—	3·0
22	M	38	P	Normal	½	Tigason	3·0
23	F	34	P	Normal	6	—	2·8
24	M	32	P+A	Normal	3	—	2·7

P = psoriasis vulgaris, PP = pustular psoriasis, A = psoriatic arthritis.

quite a lot of alcohol at this time. The reason for the second discontinuation of MTX was that serum transaminases again were greatly elevated. In the interval between the two courses of MTX, isotretinoin (Roaccutane® 20 mg, three times daily) was tried and continued for 2 months without clinical response. During the last course of MTX the serum transaminases were only slightly elevated. The cumulative MTX dose was 770 mg at the end of treatment.

Serum PIII NP levels were measured by radioimmunoassay based on the human propeptide.[12] The assay is not sensitive to the smaller degradation products of the propeptide present in human serum. Thus no serial dilutions of the sera are necessary, since the standards and the serum samples give parallel inhibition curves. The results are obtained within 3 to 4 h and the intra-assay variations are less than 5%. The reference range based on Finnish blood donors ($n = 88$) is 1·7–4·2 μg/l.

RESULTS

Nine patients (Table 1) had serum PIII NP levels above the reference range,[12] while 15 had values within the reference range (Table 2). The corresponding histological diagnoses are listed in the two tables. All but one patient with an abnormal PIII NP level had fibrosis or cirrhosis, and no normal liver biopsies were obtained from this group of psoriatics. In contrast, nine normal biopsies and two biopsies with minimal fibrosis appeared among patients with normal PIII NP levels. In one patient cirrhosis had previously been diagnosed, but thereafter in seven subsequent annual biopsies no cirrhosis was seen.[1] Two of the patients with borderline PIII NP levels (4·1 μg/l) had fibrosis. Only one patient with cirrhosis had a PIII NP level which appeared normal.

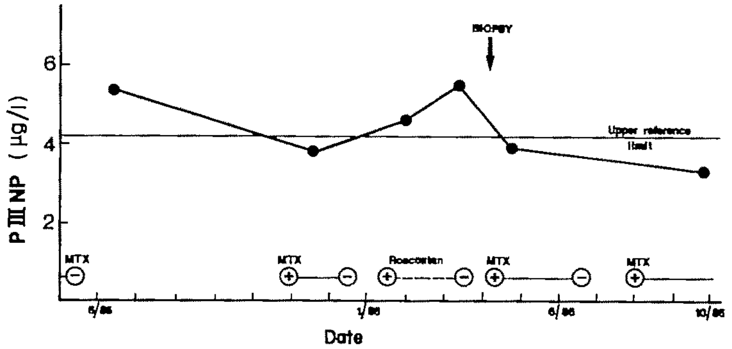

FIGURE 1. Serum PIII NP levels in relation to methotrexate and isotretinoin (Roaccutane®) treatment in a 41-year-old psoriatic patient. MTX was discontinued several times owing to elevated serum transaminases. In May 1985 the patient had a high intake of alcohol. + = started − = discontinued.

Disregarding transient increases in levels of serum transaminases, in general no abnormalities were found in other laboratory parameters related to liver function. There was no correlation between serum transaminases and PIII NP or between serum transaminases and histology. Galactose tolerance tests were all normal.

The results of six serial measurements of PIII NP levels in one patient are shown in Figure 1. These results showed elevated PIII NP immediately after discontinuation of MTX on account of a transaminase value of 368 U/l. The next elevated PIII NP values were found when the patient received retinoids. The next course of MTX was started in April 1986 after the liver biopsy had revealed only slight inflammatory changes. This treatment was discontinued again in June, since transaminases were again elevated to 143 U/l. In August 1986 MTX was restarted and at follow-up the transaminases were only slightly elevated (61 U/l). PIII NP was also not elevated at this time.

DISCUSSION

The present investigation indicates that PIII NP can be utilized to obtain a dynamic picture of processes which may lead to hepatic fibrosis in psoriatics on MTX. Our data are also in accordance with previous investigations in other pre-cirrhotic liver diseases.[10-11] There are, however, no organ-specific steps in the collagen metabolic pathway, and PIII NP is, therefore, not specific for liver fibrosis. This indicates that interpretations in individual patients should be made with caution. In theory PIII NP should not directly reflect the histology of the liver. However, we found that the more abnormal the liver biopsy findings, the higher the serum propeptide concentration. The main conclusion of our study is that if PIII NP is normal there seems to be no great risk of developing fibrosis and cirrhosis. Thus, PIII NP is a valuable non-invasive marker of fibrogenesis in the liver in this situation. It should be noted that not only MTX, but simultaneous use of other hepatotoxic agents i.e. drugs or alcohol may contribute to fibrosis and cirrhosis. The results in the patient who had six PIII NP determinations clearly indicates this. PIII NP was increased following a large consumption of alcohol and during retinoid treatment.

The present study suggests that the number of liver biopsies can be reduced to a minimum in psoriatics as long as they have normal PIII NP values. A more extensive longitudinal prospective study is now required to confirm this. As already mentioned, the advantages of our PIII NP method which make it more suitable for routine clinical use than the previously available method developed by Rohde *et al.*[10] should be noted. The present method measures PIII NP and higher molecular weight antigenic forms and is not sensitive to the degradation products which interfered in the previous method.

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Intestinal folate absorption in methotrexate treated psoriatic patients

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SUMMARY

Thirty-one psoriatic patients 14 of whom were being treated with methotrexate (MTX), participated in a controlled study to evaluate the influence of MTX therapy on the intestinal absorption of folate. Low serum and red cell folate levels in the MTX treated group confirmed previously reported findings. However, analysis of the intestinal absorption profile yielded no significant difference between the two groups. It was concluded that MTX therapy for psoriasis does not adversely affect the intestinal absorption of folate and the observed low body folate status is probably mainly the result of enhanced renal excretion.

Long-term, low dose once weekly methotrexate (MTX) therapy has proved extremely beneficial for recalcitrant psoriasis. Unfortunately, this therapy is associated with potentially serious adverse effects, principally hepatic and haematological. Low serum and red cell folate levels have been documented and in some instances symptomatic megaloblastic anaemia has supervened.[1,2] However, the precise mechanism by which MTX induces folate depletion is incompletely understood.[3] Megadoses of MTX used for the treatment of leukaemias, lymphomas and other malignancies can have a profound effect on the gastrointestinal tract resulting in nausea, vomiting, abdominal pain, gastrointestinal ulceration and haemorrhage.[4] Intermediate doses, which apparently cause no symptoms, can also provoke structural and functional abnormalities resulting in the malabsorption of drugs, amino acids and D-xylose.[5] It, therefore, seemed reasonable to hypothesize that low dose MTX therapy for psoriasis may induce folate depletion by producing intestinal malabsorption of dietary folate. The present study was undertaken to examine whether the intestinal absorption of folic acid was impaired in a MTX treated group compared with a control group of psoriatic patients.

METHODS

Patients

Fourteen consecutive psoriatic patients (10 males, four females; age range 33–74 years, mean 57 years) satisfactorily controlled on MTX (cumulative dose range 425–9, 500 mg, mean 3441 mg;

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duration of therapy, range 1–18 years, mean 7·3 years) were selected. The control group comprised 17 patients receiving topical antipsoriatic therapy and matched for sex, age and extent of psoriasis, but who had not received oral chemotherapy.

The study was approved by the local Ethical Committee and informed consent obtained from all the patients. Those patients receiving drugs or having disease known to interfere with intestinal folate absorption or to cause depletion of body folate stores were excluded from the study.

Gastrointestinal symptoms

Patients were questioned about the occurrence of the following gastrointestinal symptoms which have been described with large doses of MTX: anorexia, nausea, vomiting, weight loss, abdominal pain, diarrhoea and gastrointestinal bleeding.

Baseline investigations

The following initial tests were performed: haemoglobin, mean corpuscular volume, white cell count, platelet count, vitamin B_{12}, serum and red cell folate, urea and electrolytes, and liver function tests. All previous and current liver scans and biopsy reports of patients receiving MTX were scrutinized.

Folic acid absorption test

A folic acid absorption test was performed according to the technique of Chanarin and Bennet.[6] Body folate stores were saturated by administering oral folic acid 10 mg daily on 3 consecutive days, the last dose being given 48 h before the test. On the test day, an oral loading dose of folic acid syrup 40 μg/kg body weight was given at 0830 h, after the patients had fasted overnight. Venous blood samples were obtained via a venflon at 0, 30, 60, 90 and 120 min.

Folate measurement

For serum folate estimation, blood was collected in a plain glass tube and after 1 h was centrifuged at 1200 ***g*** for 10 min. The serum was separated by careful pipetting and stored frozen at −20°C for 3–7 days. Serum thawed at room temperature for 2–3 h was then assayed for folate using a Becton Dickinson Simul Trac-SNB Radioassay Kit with a gamma counter LKB Wallac 1270 Rackgamma II linked to a Digital Decwritten II and calculation of folate made using a Spline function (counts per min against log concentration). This radioimmunoassay technique for folate is not interfered with by the presence of MTX.

For red cell folate assay, EDTA anticoagulated blood was diluted 1:10 with 0·2% (w/v) ascorbic acid solution and incubated in the dark at room temperature for 90 min prior to freezing at −20°C. The subsequent radioimmunoassay procedure for red cell folate was the same as for serum folate.

Statistical analysis

A two-tailed *t*-test was used to compare baseline haematological parameters in the two groups. The intestinal absorption profiles were compared using the Kolmogorov-Smirnov two sample test and the Mann-Whitney U test.

RESULTS

No gastrointestinal symptoms were reported by any of the patients. Baseline haematological parameters are shown in Table 1. The only statistically significant difference between the two

TABLE 1. Baseline haematological parameters in MTX treated and control psoriatic patients. Values are means ± SEM

	Methotrexate treated group	Control Group
Haemoglobin (g/dl)	15·1 ± 1·04	14·7 ± 1·04
WBC (× 10⁹/l)	6·6 ± 1·29	6·2 ± 1·36
Platelet count (× 10⁹/l)	221·5 ± 65·4	249·6 ± 72·2
Mean cell volume (fl)	95·4 ± 6·7	90·4 ± 5·4
Vitamin B$_{12}$ (ng/l)	358·9 ± 42·5	404·3 ± 52·7
Serum folate (μg/l)	2·2 ± 1·69*	3·7 ± 1·8
Red cell folate (μg/l)	130·2 ± 109·9*	220·2 ± 109·6

* Significantly lower than control group, $P < 0{\cdot}05$; two tailed *t*-test.

groups was in the serum and red cell folate levels which were significantly lower in the MTX treated group ($P < 0{\cdot}05$). Urea and electrolytes were within the normal range in all the patients. However, two patients in the MTX group had a mild isolated elevation of alanine aminotransferase, but normal liver biopsies; two other patients had mild to moderate fatty infiltration on liver histology, but normal biochemistry. Liver function tests were normal in all the other patients and liver scans and biopsy results were also unremarkable in the other MTX treated patients.

The intestinal folate absorption profiles are shown in Figure 1. The predominant absorption of folate occured rapidly within the first half-hour and a plateau was reached approximately one hour following oral loading. However, the parameters of intestinal absorption, including maximum rate, peak and total absorption, did not differ significantly between the two groups of psoriatic patients, despite the MTX group having a lower basal serum folate level.

DISCUSSION

The present study confirms the previously reported finding of low serum and red cell folate levels in psoriatic patients receiving MTX therapy. However, this deficiency was not of sufficient severity to produce a megaloblastic anaemia nor to significantly raise the mean corpuscular volume. The mechanism by which MTX induces folate depletion has been studied in the Wistar rat using radiolabelled folic acid; increased renal excretion, together with intestinal malabsorption and reduced hepatic storage of folate, have been observed.[7] Studies in man have also shown an adverse influence of MTX on intracellular folate storage. Body folate is present predominantly in the form 5-methyl tetrahydrofolate[8] and can only be stored intracellularly following conjugation by the enzyme pteroyl-glutamyl conjugase.[9] However, MTX also acts as substrate for this enzyme with the formation and storage of MTX polyglutamates.[10] Therefore, less folate will be conjugated and stored intracellularly in the presence of MTX.[11] This accounts for the observed reduction by MTX of red cell folate but not for the diminished serum folate level. The latter may arise as a result of enhanced renal excretion or intestinal malabsorption of folate or both.

We studied the influence of MTX on the intestinal absorption of folate, as MTX used in large doses for the treatment of a variety of malignancies has been shown to have a deleterious effect on the structural and functional integrity of the small intestine. Lethal doses of MTX in

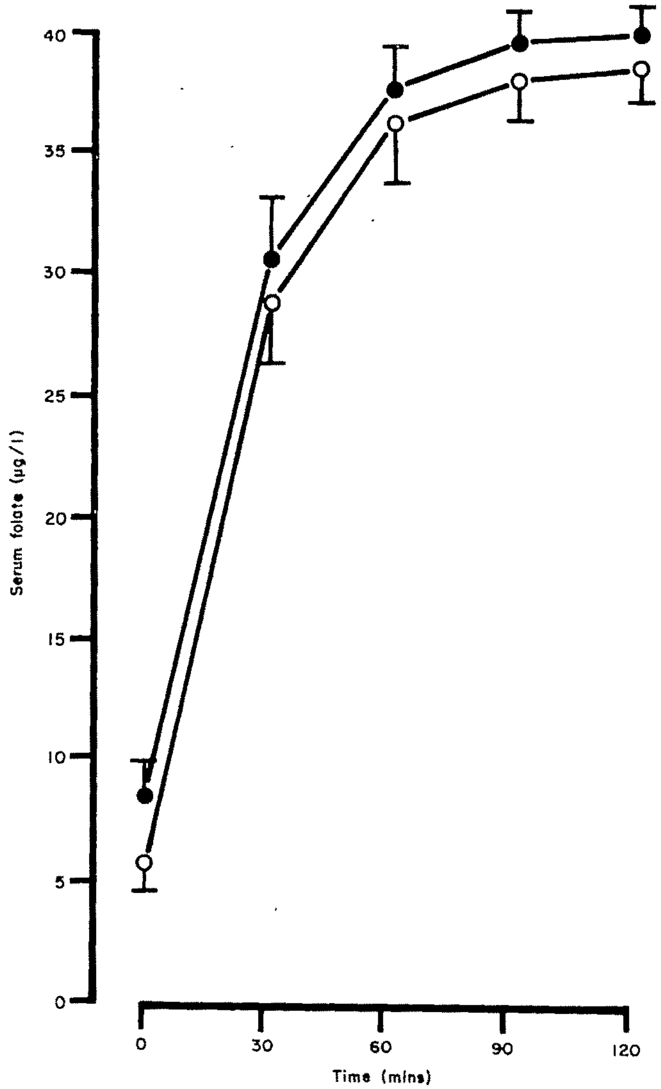

FIGURE 1. Serum folate levels in MTX treated (○) and control (●) psoriatic patients. Points are means ± SEM for 14 MTX treated patients and 17 controls.

experimental animals may produce desquamation of the intestinal mucosa[12,13] and doses used in humans can also produce dramatic gastrointestinal symptoms necessitating leucovorin rescue therapy. Lower doses of MTX which are asymptomatic can produce morphological changes in the human proximal small intestine[14,15] with concomitant malabsorption of amino acids and drugs, including phenobarbitone, isoniazid and MTX itself.[4,5,16] Kamen *et al.*[3] suggested MTX induced intestinal malabsorption to explain folate-depletion in their patients with leukaemia. Animal studies have yielded data in support of folate malabsorption induced by MTX but the dose of the drug used was excessive.[7,17] Freedman *et al.*[18] attempted to evaluate the impact of MTX therapy in psoriatic patients on the intestinal absorption of folate using a tritiated folic acid urinary excretion test. In this test the urinary excretion of radiolabelled folic acid was measured following oral ingestion and results compared between psoriatic patients and normal volunteers. An increased urinary excretion of radiolabelled folate was observed in the MTX treated group and this was interpreted as indicating that intestinal absorption of folic acid was unimpaired by low dose MTX. Unfortunately, the design of this study does not allow the renal and intestinal effects of MTX to be differentiated. We, therefore, used a more direct approach to measure intestinal folate absorption, but our study also demonstrated that no significant malabsorption was caused by MTX.

Thus, MTX induced folate depletion in psoriatic patients is unlikely to be due to significant intestinal malabsorption and is probably mainly the result of enhanced renal excretion. Folate filtered by the glomerulus is mostly actively reabsorbed by the renal tubule and inhibition of this process by MTX[19] would augment urinary loss and diminish body folate stores.

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Objective measurement of three epidermal parameters in psoriasis vulgaris and in dermatopathology in general

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SUMMARY

Three objective parameters which characterize the main features of the epidermis are described. One parameter relates to the mean thickness of the epidermis determined by a simple method. The second descriptive number is the ratio of the thickness of the strata corneum, lucidum and granulosum to the stratum malpighii. The third number relates to the degree of folding of the dermo-epidermal junction.

The ratio of the mean thickness of abnormal to normal stratum corneum (for the same skin region) is suggested as an objective measure of hyperkeratosis; likewise, the ratio of abnormal to normal stratum malpighii is an objective measure of acanthosis.

A single parameter can be measured against time to follow changes during the pathogenesis, treatment or remission of epidermal disease.

As psoriasis undergoes progressive acanthosis, the geometry of the folding of the dermo-epidermal junction is such that cells of the stratum malpighii retain their close association with the cylindrical dermal papillae.

The human epidermis varies morphologically with age, body region and pathology. In other mammals there are often marked seasonal changes to take into account.[1] Some of the parameters are the mean thickness, the convolutions of the dermo-epidermal junction and the ratio of the thickness of the strata corneum, lucidum and granulosum to the rest of the epidermis.

Computer-assisted image analysis allows easy and objective measurement. Some or all of these measurements can be related in a single graph. A single point on this graph or a set of three numbers, therefore, characterizes the epidermis for any body location. Furthermore, these parameters can be measured repeatedly to record changes during the development and resolution of diseases of the epidermis.

A simpler version of this approach has been used in a study of psoriasis.[2] In the present study, psoriasis vulgaris was monitored during treatment as an example of this approach.

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METHODS

A 22-year-old white female with a history of recurrent psoriasis received daily outpatient treatment by the Ingram regime.[3] Two millimeter punch biopsies were taken, using a disposable punch, before treatment and at intervals during treatment, from the same plaque on the upper lateral aspect of the left arm and from nearby normal skin for a control. The specimens were fixed immediately in phosphate buffered formaldehyde, washed in 70% ethanol and stained for 10 min in alcoholic eosin to allow accurate orientation during sectioning. The tissue was dehydrated in ethanol, cleared in xylene and embedded in paraffin. It was sectioned at 14 μm, dewaxed and stained in eriochrome cyanin[4] (which resembles haematoxylin and eosin) and mounted.

A Zeiss IBAS 1B Semiautomatic Image Analysis Computer System was used to analyze a photographic or TV image of the sections.

The mean epidermal thickness parameter

The simple method used to determine the mean epidermal thickness was to take the epidermal area over a set distance then divide this area by the same distance thus arriving at the mean thickness (Fig. 1). The mean thickness of any stratum within the epidermis can be calculated in the same manner. Any islands of papillary dermis in the epidermis were subtracted from the total area before division by the distance. If the skin section is not flat, but is instead curved, then the area must be divided by the length along the superficial side of the stratum spinosum.

If the embedded biopsy is not properly orientated with respect to the knife then the epidermis will be cut at a slant and hence appear thicker. The slant error can be calculated according to Figure 2.

If T_t is the true thickness, T_e, the erroneous thickness and e°, the misalignment angle then

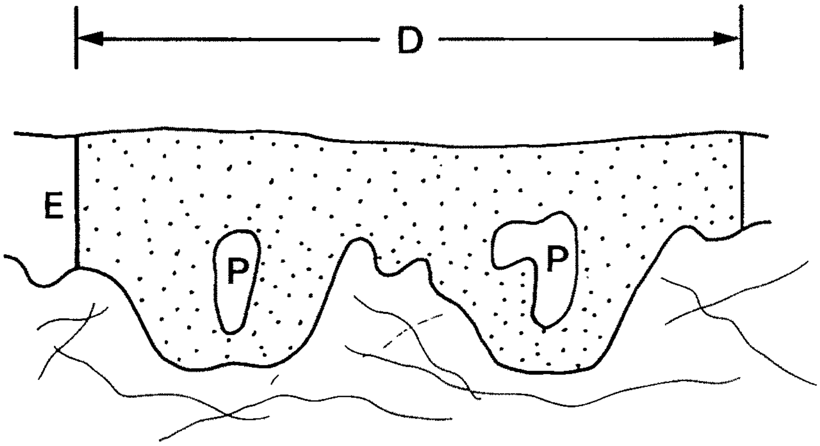

FIGURE 1. Calculation of mean epidermal (E) thickness and the degree of folding of the dermo-epidermal junction. The mean thickness (T) is found by measuring the stippled epidermal area (A) over the distance (D); this area is then divided by D, (A/D = T). The areas of the isolated dermal papillary patches (P) are not included in A. The degree of dermo-epidermal folding is found by measuring the length of the dermo-epidermal junction over the distance (D), adding this to the perimeters of the isolated dermal papillae (P) then dividing this sum (J) by D, (J/D).

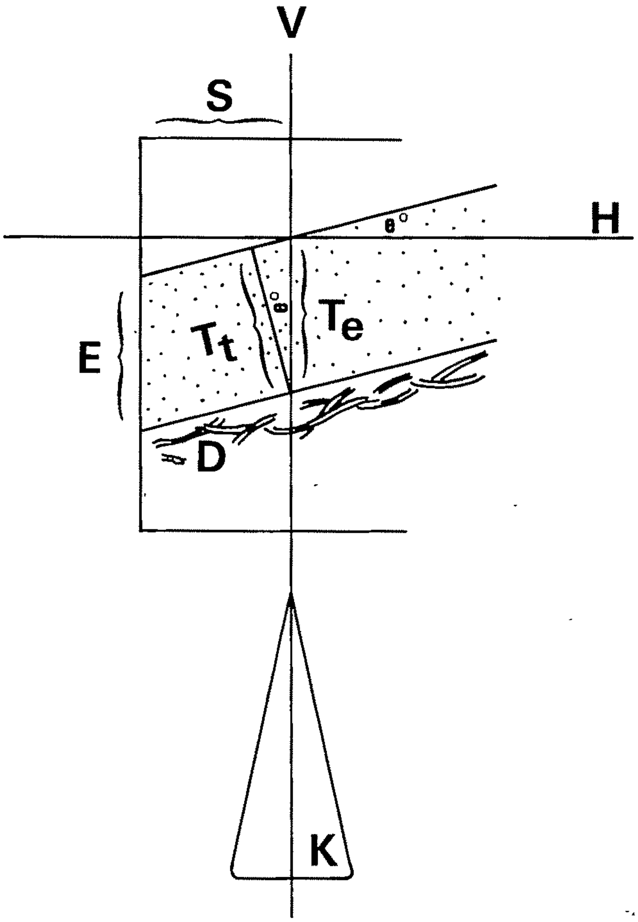

FIGURE 2. Calculation of the amount of error in the thickness of the epidermis in a misaligned piece of embedded skin. Side view of paraffin block and knife. See text. D, dermis; E, epidermis; e° misalignment angle; H, horizontal; K, knife; S, section thickness; T_e, erroneous thickness; T_t true thickness; V, vertical.

$T_e = T_t \sec e°$. Since T_t is a constant then T_e varies as sec e°. The relationship between e°, sec e° and percentage error is shown in Table 1. (Sec = secant, a trigonometric function).

Block orientation is, therefore, not a significant source of error because the block can be orientated within 5° by eye.

The keratinized: keratinizing parameter

Another characteristic feature of the epidermis is the ratio of the thickness of the last stages of kertinization (strata corneum, lucidum, and granulosum) to the early stages of keratinization (stratum malpighii). Of more practical interest to the dermatopathologist is a modification of this idea to measure hyperkeratosis and acanthosis.

In hyperkeratosis the stratum corneum is abnormally thick. An objective measure of this condition (H) is arrived at by dividing the mean thickness of the abnormal stratum corneum (C_a) by the mean thickness of normal stratum corneum (C_n) hence $H = C_a/C_n$. The normal control

TABLE 1. Relationship between the misalignment angle of the embedded epidermis, its secant and the percentage error increased area presented in the section

e°	sec e°	% error
1	1·00015	< 1
5	1·00382	< 1
10	1·01543	1·5
15	1·03528	3·5

tissue is taken from nearby healthy skin or from healthy skin on the corresponding opposite side of the body. This measure was not used in the present study because of the difficulty in retaining the stratum corneum.

In acanthosis the stratum malpighii is abnormally thick. As for hyperkeratosis, the analogous measure for acanthosis (A) is M_a/M_n where M_a is the mean thickness of the abnormal stratum malpighii divided by the mean thickness of the same stratum in normal skin (M_n).

The value, A, varies slightly from serial section to serial section because the dermal papillae are either waxing or waning; however, these variations compensate for each other in an extensive section or series of sections.

The degree of dermo-epidermal contact parameter

The length of the dermo-epidermal junction over a set distance can be measured. The perimeter of any dermal papillary 'islands' must be added to arrive at the total junction length (Fig. 1). The degree of folding of this junction is J/D where J is the length of the junction and D is the straight distance over which the measurement is made. If the skin is curved then this distance is measured along the superficial border of the stratum spinosum.

Use of the descriptive values

The mean thickness of the stratum malpighii, degree of acanthosis and dermo-epidermal fold ratio can be all combined in a three-dimensional graph in order to integrate these three variables of the acanthosis of psoriasis.

Depending on the dermatosis, some parameters will be of more significance than others. The significant parameters can be plotted against time in order to detect changes during pathogenesis or therapy.

From each biopsy, four widely spaced sections were measured over the full width as described above. The reproducibility error of the measurements was calculated by measuring the same section 10 times, then expressing the standard deviation as a percentage of the mean.

Determination of the shape of the dermal papillae

The shape of the dermo-epidermal interface from florid psoriatic plaques from two male patients, (Patient 1 aged 48 and Patient 2 aged 56) was determined by the split skin technique and scanning electron microscopy. Comparison was made with clinically normal nearby control skin from the patients. Because the control skin in Patient 1 had received extensive UVB treatment, lesional skin from the lumbar region was compared with skin from the same region from one of us (D.M.C., aged 52).

The biopsies were soaked at room temperature for 1 h in a lithium thiocyanate solution made as follows (D.M.Chapman, unpublished). Twenty millilitres of 26% (4M) LiSCN in distilled water were brought to near neutrality with drops of concentrated HCl. To this was added 20 ml of M/15 Sörensen's phosphate buffer at pH 6·8. Fine forceps were used to peel off the epidermis. The separated epidermis and dermis were fixed for a few days in phosphate buffered formaldehyde. The fixative was washed out in normal saline and the specimens then post-fixed in 1% OsO_4 in normal saline for 1 h. The pieces were dehydrated in an ethanol series, critical point dried, glued with silver glue to a stub, gold coated and viewed by scanning electron microscopy. The thin type of epidermis should be glued to a piece of cover glass with cyanoacrylate glue before the post-fixation otherwise the tissue curls up and cracks when attempts are made to flatten it after critical point drying.

Another pair of biopsies from Patient 2 was fixed and post-fixed as above before embedding in Taab (Reading, U.K.) resin for light microscopy. One specimen was sectioned at 1·5 μm in the usual manner perpendicular (radial) to the surface; the other was sectioned tangential (parallel) to the surface in order to reveal transverse sections of the dermal papillae. This resin gives minimal shrinkage. The sections were stained in 1% toluidine blue in 1% borax and mounted.

The tangential sections from different levels were measured to determine the mean diameter of the dermal papillae and the diffusion distance from the edge of the dermal papilla to the farthest cells in the stratum malpighii. This diffusion distance was determined by measuring the area of the whole section (Fig. 3a). From this area the areas of the dermal papillae were

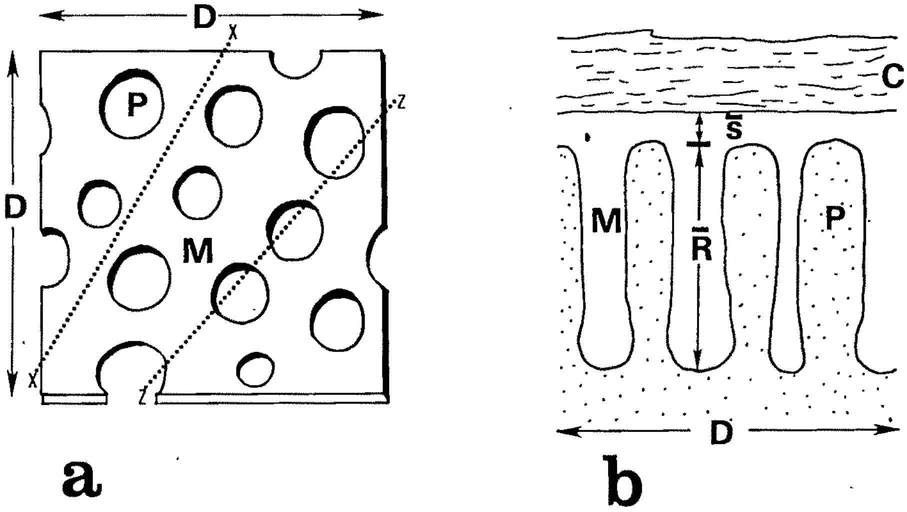

FIGURE 3. Parameters involved in an alternative method for measuring the thickness of the psoriatic stratum malpighii. See text. (a) Tangential section of the stratum malpighii. (b) Perpendicular section of the same skin. C, stratum corneum; D, distance along the section or photograph; M, stratum malpighii; P, dermal papilla; $\bar{R}$, mean length of the rete pegs; $\bar{S}$, mean thickness of the suprapapillary plate of the stratum malpighii. The perpendicular planes of section (x – – – – – x) and (z – – – – – z) show how, in a small sample, a section can either miss the dermal papillae or section many of them.

subtracted to arrive at the area of the stratum malpighii. Next, the sum of the circumferences of the dermal papillae was measured; those papillae at the edge of the section had only part of their circumferences present. The sum of the circumferences divided into the area of the stratum malpighii gave a value for the mean diffusion distance. This distance was used as a unit length in constructing a model of the psoriatic stratum malpighii.

As shown below, a model representing the psoriatic stratum malpighii is much simpler than the complicated shape suggested by perpendicular sections.

A test to determine whether dermal papillae are randomly arrayed

The disposition of cross sections of dermal papillae in a tangential section of the stratum malpighii did not present a regular geometric array. To determine whether or not the array was random, the Poisson distribution was compared with the observed distribution of dermal papillae in a 20 × 20 cm photograph at a magnification of × 360. A tracing paper overlay of the same size and ruled into 16 5 × 5 cm squares was used to record the number of dermal papillae per square. A dermal papilla was assigned to the square which contained the majority of the dermal papilla's area. The significance of the difference between the observed and Poisson distribution was checked by the chi-square test.[5]

Another more complicated but more exact method of calculating the mean thickness of the psoriatic stratum malpighii

The cylindrical shape of the psoriatic dermal papillae permits another method of calculating this mean. The problem with the first method is that a perpendicular section could go between many dermal papillae and thus overestimate the mean thickness (Fig. 3a). The other method circumvents this problem, but has the drawback of requiring both a perpendicular and a tangential section of the same skin region. With reference to Figure 3a, $A_w - A_c = A_m$, where A_w is the area of the whole square section, A_c is the sum of the areas of the dermal papillae profiles and A_m is the area of the stratum malpighii tissue.

$D^2 - A_c = A_m$, where D is the length of the square tissue section.

$V_w - V_c = V_m$, where V is the volume and the subscripts are as above.

Because volume = area × thickness, the volumes can be expressed as:

$$D^2\bar{R} - A_c\bar{R} = V_m,$$

where $\bar{R}$ is the mean length of a rete peg (Fig. 3b).

The mean thickness, $\bar{T}$, of the part of the stratum malpighii containing dermal papillae is:

$$\bar{T} = V_m/D^2 \text{ (Thickness = volume/area)} = (D^2\bar{R} - A_c\bar{R})/D^2 = \bar{R}(1 - A_c/D^2)$$

The total thickness of the stratum malpighii is $\bar{T} + \bar{S}$. $\bar{S}$ is the mean thickness of the suprapapillary part of the stratum malpighii (Fig. 3b) as measured from perpendicular resin sections. This suprapapillary plate is quite thin in psoriasis.

RESULTS

Figure 4 is a three-dimensional graph showing the relationship of the three parameters detailed in Table 2. This complex graph is not complicated by confidence limits or other statistics; these can be obtained from Table 2. The reproducibility error of the image analysis measurements is less than 1%.

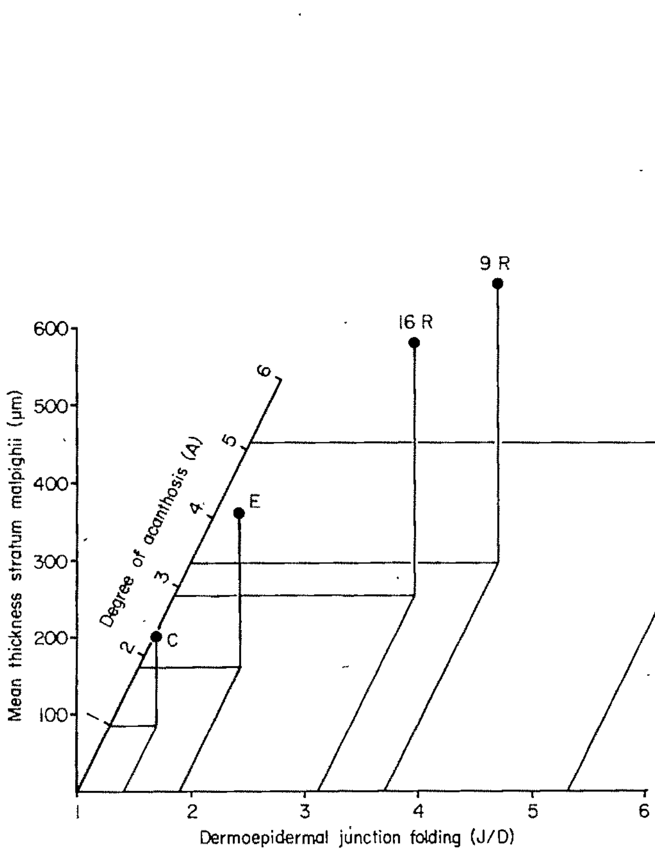

FIGURE 4. Three-dimensional graph incorporating three parameters measured during the course of treatment of psoriasis vulgaris. See text. C, control healthy skin; E, early stage of disease; O R, advanced stage without any treatment; 9 R, 16 R: 9 and 16 days of treatment, respectively.

These three parameters are plotted against time in Figure 5. Levels of all parameters were high in the florid example of psoriasis and all decreased with treatment.

When the mean thickness of the stratum malpighii is plotted against the degree of dermo-epidermal folding (data from Table 2), the result is a straight line. This means the folding keeps up with the thickening. Since diffusion of oxygen and nutrients from the papillary layer of the dermis into the stratum malpighii starts out from the dermo-epidermal junction, it is hypothesized that the geometry of the folding of this junction must be such that a thickening of the stratum malpighii does not result in any epithelial cells being too remote from the dermis. The model for this geometry is developed below.

The approximate shape of the dermal papillae as seen by scanning electron microscopy (Fig. 6) turns out to be simple cylinders poking into corresponding holes in the epidermis. This simple geometry is not apparent from perpendicular sections.

For comparison, nearby control skin was studied. Unfortunately, this skin was not completely normal because UVB treatment had caused some ambiguous features characteristic of mild psoriasis. The UVB-induced erythema could account for the mild inflammatory response

TABLE 2. Measurements of normal and psoriatic epidermis

Biopsy type	Stratum malpighii thickness μm	Degree of acanthosis	Degree of folding of dermo-epidermal junction (J/D)
Florid lesion, before treatment	560(20) ± 30	5·1	5·3(0·2) ± 0·3
Same lesion after 9 days treatment	360(20) ± 30	3·3	3·7(0·4) ± 0·6
Same lesion after 16 days treatment	320(10) ± 15	2·9	3·1(0·3) ± 0·5
A different early lesion	200(20) ± 30	1·8	1·9(0·3) ± 0·5
Healthy nearby control skin from same patient	110(10) ± 15	1·0	1·4(0·1) ± 0·2

Values are means (standard deviations) ± 95% confidence limits of four measurements per biopsy.

(dilated capillaries) and slight acanthosis which are psoriasiform features. The significant difference is that the control dermal papillae are short blunt cones.

Non-psoriatic skin from Patient 1 was compared with skin from a normal subject. The patient's skin was judged to have 'slight acanthosis.' Acanthosis can be expressed objectively as follows. The thickness of each subject's stratum malpighii was averaged from three widely spaced sections; Patient 1, mean 65 μm, S = 3, normal subject (D.M.C.) mean 45 μm, S = 3.

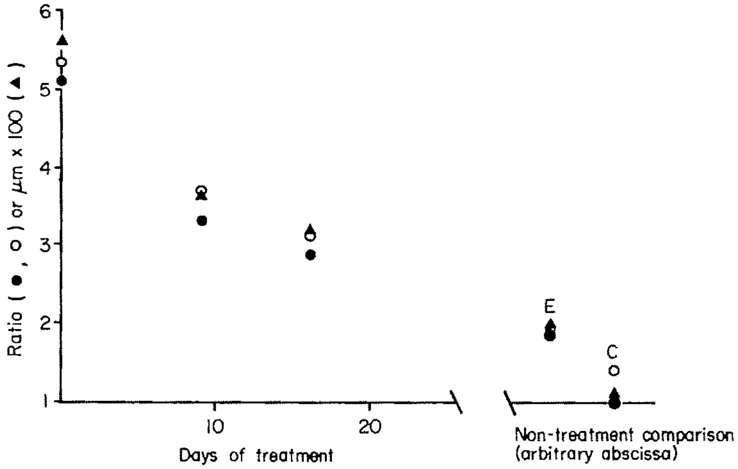

FIGURE 5. Changes in the degree of acanthosis (A) (●), degree of folding of the dermo-epidermal junction (J/D) (○) and the mean thickness of the stratum malpighii (▲) during treatment for psoriasis vulgaris of the arm. This is compared with nearby control healthy skin (C) and a nearby early lesion (E).

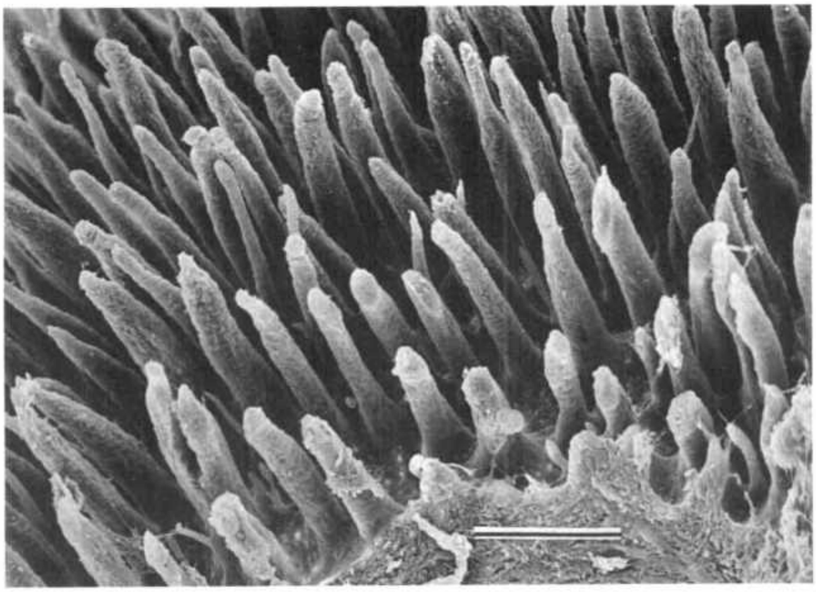

FIGURE 6. Scanning electron micrograph of the lumbar dermis from a split skin preparation showing the dermal papillae sticking up from the dermis. Florid psoriatic plaque from a male patient (Patient 1). Bar = 200 μm.

These means are significantly different ($P < 0.05$). The degree of acanthosis in non-psoriatic skin from Patient 1 is $65/45 = 1.4$.

The mean diameter of the psoriatic dermal papillae at three widely separated levels is almost the same ($\bar{Y} = 65$ μm; $S = 3$). At any level the diameters of the papillae were, however, more variable ($S = 13$; $n = 24$).

Whether dermal papillae are randomly arrayed

There were 17 dermal papillae on the test photograph spread out over 16 squares, giving a mean of $17/16 = 1.0625$. One square had no papillae, 13 squares had one and two squares had two papillae. When these data were compared with the Poisson distribution and the differences tested by the chi-square technique, it was found that the dermal papillae are not randomly arrayed. Even though the array is not geometrically regular, nevertheless, the dermal papillae are almost evenly spread out.

Fusion of dermal papillae is to be expected in a random distribution of dermal papillae, but this seldom happens. Fused papillae can be seen in scanning electron micrographs and in cross sections of dermal papillae where fused papillae have a figure-eight shape. In the deepest region of the epidermis more fusion occurs.

Data for a model of the psoriatic stratum malpighii

The mean thickness of the stratum malpighii above the tips of the dermal papillae is 43 μm ($n = 18$, $S = 19$). Since there is no stratum granulosum in psoriatic skin, the border of the stratum spinosum is taken to be where the cells lose their polyhedral shape and become flatter and darker.

Figure 3a is the type of preparation analysed to determine the spinosum cells farthest from the dermo-epidermal junction. The same three levels, as in four paragraphs back, show a diffusion mean distance of 49 μm (S = 1).

The model for the psoriatic stratum malpighii based on these data suggests a unit structure which approximates to a tube closed at one end. The walls are 49 μm thick, the lumen is 65 μm wide and the end cover is 43 μm thick. No matter how long the tube grows, its walls remain the same thickness to allow for nutrient diffusion.

DISCUSSION

The calculation of the thickness of the epidermis might at first thought seem difficult because the dermo-epidermal junction is often wavy. The usual way to solve the problem of computing this mean is by measuring and averaging the thickness at many random intervals; the larger the sample of measurements, the more accurate the mean. The present method, made possible by the area mode of the image analyser, is much easier to perform and is more accurate. This eliminates the subjective guesswork often encountered in descriptive dermatopathology.

Since the epidermis varies from region to region, any study must stipulate the body region under investigation. There may, by chance, be normal skin nearby or on a corresponding region on the other side of the body to serve as a control. We do not recommend comparing a patient's abnormal skin with someone else's normal skin from the same region as was done in the didactic example in the present study; however, there are dermatoses in which the patient has no normal corresponding skin for comparison and this is the only approximate comparison open to the investigator.

Some difficult epidermal sectional geometries such as the reticulated acanthotic form of seborrhoeic keratosis, the development of pseudoepitheliomatous hyperplasia and the changes in the epidermal layers in the various congenital ichthyoses could be measured easily by this method.

There may be artifactual or real difficulties in the measurement of the stratum corneum. The histological artifact arises from the easy separation of this stratum from the rest of the epidermis. The real difficulty arises in dermatoses like psoriasis vulgaris where large flakes of corneum are easily shed. This will give either a high or low degree of hyperkeratosis before or after shedding, respectively, and would affect determinations of the thickness of the whole epidermis.

There is a serious theoretical drawback to this metric approach because a perpendicular section going between many dermal papillae as opposed to going through many papillae (Fig. 3a) would give very different values for all three parameters. To correct this, the sample size is increased by having extensive sections or by measuring many sections from different depths in the block.

The LiSCN method for splitting skin has not been examined with any rigour; however, the scanning electron microscope results seem compatible with the section measurements even though the solution is hyperosmotic. An exception to this compatibility relates to the deep ends of the sectioned rete pegs which are often round in psoriasis (Fig. 3b), yet in Figure 6 the bases of the dermal papillae in the scanning electron micrograph do not show a corresponding constriction. It is possible that the soft dermal papillae are deformed as they are pulled from their epidermal sockets.

The randomization test shows that the dermal papillae are not randomly arrayed, yet neither are they arranged in any special geometric plan. The shape and array of the dermal papillae is such that no living epidermal cell is too far from its nutrient source in the dermis.

It is, therefore, possible to describe approximately a skin region's normal or abnormal epidermis using three numbers. Numbers, of course, can be manipulated mathematically unlike verbal descriptions. It would be of interest to monitor these parameters for certain dermatoses over time during the pathogenesis, natural remission and treatment to determine if all three overlapped or took different routes on the graph. This might give insight into the mechanisms of a disease process.

In a chapter entitled 'The case for morphometry in diagnostic pathology', Baak and Oort[6] argue for morphometry's advantages of objectivity, reproducibility and detection possibility. By 'detection possibility' they mean that differences which escape subjective assessment can be detected by quantitative means and thus lead to a diagnosis. Baak and Oort[6] list over a dozen organs and show how pathologists' subjective assessment of the degree of pathology can be as much as 87% in disagreement. These authors do not include skin in their list, but the argument holds for this organ as well.

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The symptomatology of hidradenitis suppurativa in women

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SUMMARY

In order to establish the possible role of androgen in the development of hidradenitis suppurativa the symptomatology of the disease was studied in a group of 70 female patients. The results were compared with those obtained from an age-matched control group of healthy women.

The incidence of women with signs of androgenization in the two groups did not differ significantly. The only significant differences found were a shorter menstrual cycle and a longer duration of menstrual flow in the women suffering from hidradenitis, and that women with hidradenitis were more likely to have a positive family history of hidradenitis. The prevalence of hidradenitis in the control group was 4%.

The results show that hidradenitis is not accompanied by other signs of androgenization. The disease may be due to local changes in the aprocrine glands of predisposed individuals.

Hidradenitis suppurativa is a disease of the apocrine glands, occurring predominantly in women and causing considerable morbidity. Although a number of studies have been made of the disease the aetiology remains obscure. It has been shown that a significant degree of hyperandrogenism can be measured in female patients with hidradenitis[1]. Studies of families have suggested a genetic predisposition.[2] The use of depilatories, deodorants, talcum powder and shaving have frequently been suggested as causing the disease, but the use of these by patients with hidradenitis has been shown not to differ significantly from that in a matched group of healthy controls.[3]

In order to establish the possible influence of androgens in patients with hidradenitis suppurativa, information on other symptoms of androgenization was obtained from a group of female patients with hidradenitis suppurativa.

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METHODS

Patients referred for surgical treatment of hidradenitis suppurativa by either general practitioners or practising dermatologists, were systematically questioned about possible symptoms of cutaneous virilization, menstrual cycle, pregnancy, and family history. The diagnosis of hidradenitis suppurativa was based on the clinical presentation at the time of the operation, and a history of recurrent and chronic inflammation, recurring at the same place in the groin, under the arms, between the buttocks or on the breasts, the lesions suppurating, and unresponsive to normal treatment for boils, such as lancing.

Subjective scoring systems for acne[4] and menstrual cycles[5] are reported to be reliable. The data on menstrual cycles was compared with previously published data.[5] Data on acne and hirsutism was condensed into a yes or no answer and no grades were recorded. Information obtained from the patients was supplemented by information taken from the patients' records.

A control group was chosen from female hospital staff and from patients referred for cosmetic excision of naevi or warts. None of the women in the control group had any personal history of hidradenitis suppurativa or any other infection involving the axillae, submammary regions or the groin. Information on menstrual cycles was compared with data from previous studies of the general population[5] in order to have as large a control group as possible.

Student's t-test and Fischer's exact test were used for statistical analysis.

RESULTS

Information was obtained from 70 women (mean age 31 years, range 17–59 years) suffering from hidradenitis suppurativa. The mean age at onset of the disease was 23 years (range 6–58 years). The distribution of the ages of onset of the disease is shown in Figure 1. The localization of the hidradenitis is shown in Figure 2; of the 70 patients 49 had disease in one region only. The lesions were residual, resistant lesions after medical therapy. Not all patients were able to give complete answers, but they did not differ from the remainder of the group.

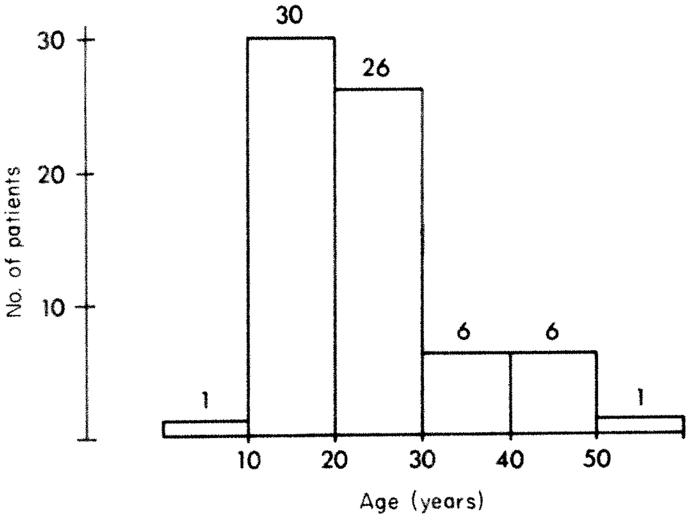

FIGURE 1. Age at the onset of hidradenitis suppurativa.

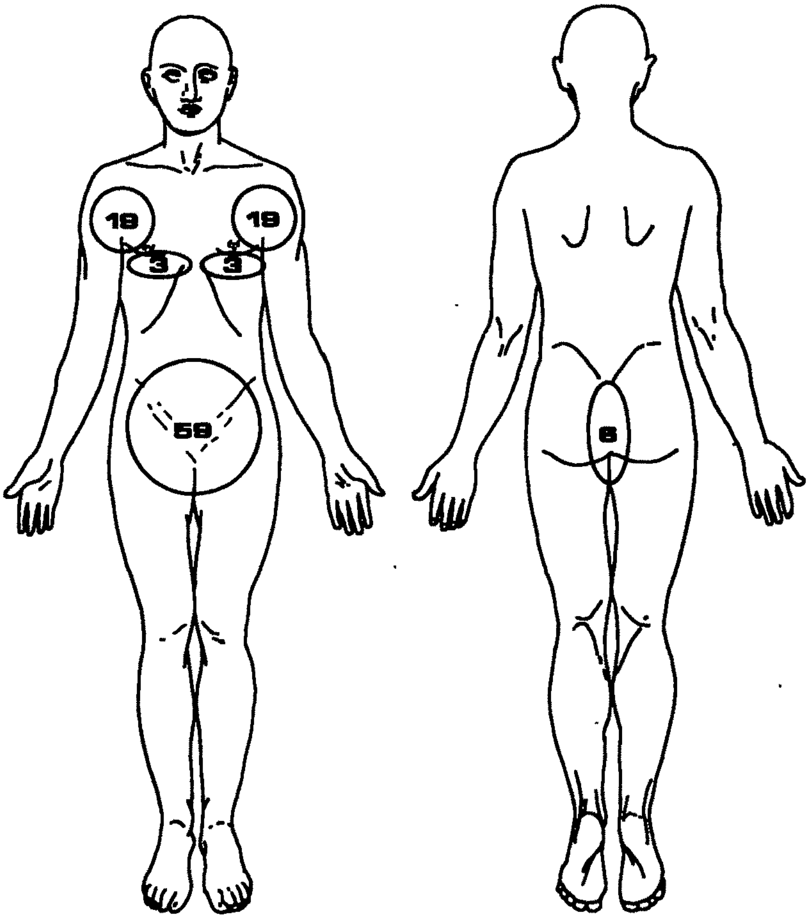

FIGURE 2. The localization of the lesions of hidradenitis suppurativa. Numbers indicate numbers of patients with lesions at each site. Some patients had lesions at more than one site.

An age-matched control group of 100 women was selected at random from healthy hospital staff or patients referred for cosmetic excisions of naevi or warts. As the patients studied were all in good general health apart from their hidradenitis, the control group was not in any other way different from the group of patients studied.

In the control group 4 women were found to have hidradenitis suppurativa and were transferred to the study group. This would suggest a prevalence of approximately 4% in the general female population.

No significant difference was found in the number of women complaining of acne, hirsutism, or irregular menstrual periods in the two groups (Table 1).

Eighteen of the 70 patients had a positive family history of one or more cases of hidradenitis. This was significantly more than in the control group where only two of 96 subjects had a positive family history of hidradenitis ($P < 0{\cdot}001$).

The menstrual cycle in the patients with hidradenitis was found to be significantly shorter than that of the general female population between 23 and 45 years of age ($P < 0{\cdot}001$) and the

TABLE 1. Numbers of patients and controls complaining of acne, hirsutism or irregular periods, and duration of the menstrual cycle

			Patients	Controls	*P*
Acne	Face	Yes	22	30	NS
		No	45	65	
	Back	Yes	14	11	NS
		No	52	78	
Excessive growth of hair	Face	Yes	3	5	NS
		No	61	85	
	Chest	Yes	1	3	NS
		No	61	85	
	Legs	Yes	20	19	NS
		No	48	77	
Irregular periods		Yes	4	8	NS
		No	43	59	
Length of menstrual cycle (days) (mean ± SD)			26·1±3·2	28·9±0·4*	<0·001
Duration of menstrual flow (days) (mean ± SD)			5·4±1·4	5·0±0·1*	<0·05

Not all subjects answered all questions.

* Control values taken from previously published study.[5]

TABLE 2. Changes in the severity of hidradenitis suppurativa during and after pregnancy

	No. of patients			
	Better	Unchanged	Worse	Total*
During pregnancy	9	17	6	32
Post-partum	2	15	11	28

* Three patients were pregnant at the time of interview.

duration of the menstrual flow was found to be significantly longer in patients with hidradenitis ($P<0{\cdot}05$). Thirty-three of 65 patients experienced exacerbation of the disease during menstruation, two noted improvement during menstruation and 30 had found no change during the menstrual cycle. The influences of pregnancy and childbirth are shown in Table 2.

DISCUSSION

Hidradenitis suppurativa is an inflammatory disease of the apocrine glands. It occurs more frequently in women[1,6,7] than in men, and it has been suggested that axillary disease predominates in women. The present study suggests that genito-femoral disease is also more common in women.

Experimental evidence suggests that poral occlusion[8] is essential to the pathogenesis of the disease, and the presence of retroauricular comedones has been suggested as a diagnostic sign of hidradenitis.[1] Several authors[6, 9–12] have also suggested that acne may be part of the clinical picture of hidradenitis although later studies have only found acne in 45% of patients with hidradenitis.[1] Other studies have mentioned an unspecified incidence of acne or acne scars, and some cases have been reported in which hidradenitis occurred together with acne conglobata.[13,14] This in turn has led to therapeutic trials of anti-androgens in hidradenitis,[15] but although a number of patients have been helped, the response has not been complete. This suggests that other factors may be important in the development of the disease.

The results of the present study suggest that the number of women with hidradenitis complaining of symptoms of cutaneous virilization is not significantly different from that among age-matched healthy controls. Generally the role of androgens in hidradenitis seems unclear.[16] Men are afflicted much less frequently than women, and there are contradictory reports some stating that the male sex hormone has caused flares of hidradenitis[7,17] and others in which it has been used successfully for the treatment of the condition.[18]

Some form of hormonal influence on the course of the disease is, however, strongly suggested by the natural course of the disease and the cyclic variations coinciding with the menstrual cycle. The disease becomes progressively rarer with age. During periods where there are high levels of oestrogens the disease is ameliorated, while menstrual flow, which reflects falling levels of oestrogens, is accompanied by a flare of the hidradenitis. Menstrual flare was noted by a major proportion of the patients in the present study. In the present study the only significant differences regarding the menstrual cycle in hidradenitis patients were found to be a shorter cycle and a longer period of menstrual flow. The number of women complaining of menstrual irregularities did not differ significantly from that in the control group. The longer menstrual flow and shorter menstrual cycle seem to be the only signs of an affected, pituitary-ovary-axis in the women suffering from hidradenitis. It is possible that the relatively longer menstrual flow represents periods of relative hyperandrogenism in these patients, or it may be that these changes are due to inherent differences of steroid-receptor dynamics in different individuals. At present this remains unexplained.

Pregnancy and childbirth are also reported to influence the course of the disease,[1,19] with improvement during pregnancy and post-partum flaring. The present findings seem to confirm this general trend, although it was not the case in all the patients. The changes occurring during pregnancy have previously been ascribed to the high levels of oestrogens in the blood, but in view of the many general changes associated with pregnancy other explanations may prove to be as important.

On the basis of family studies a genetic predisposition to hidradenitis has been suggested, possibly involving autosomal dominant inheritance.[2,20,21] The exact prevalence of the disease is unknown, but has been estimated at 1 in 3000. The results obtained in the present study suggest a substantially higher prevalence of approximately 4% in the female population. A highly significant proportion of the patients studied had a positive family history when compared with the controls, supporting the hypothesis that a genetic factor may be important in the aetiology of hidradenitis. Such a genetic predisposition may offer an explanation of the confusing role of androgens in hidradenitis. It is possible that the increased incidence in families is due to a genetically determined end-organ malfunction, which is only manifest in certain circumstances, thus making any manifestation of the disease dependent on concurrent internal and external factors.

The results of the present study suggest that hidradenitis suppurativa in women is due to local changes in the apocrine glands of predisposed individuals and that a positive family history and a clinical picture of prolonged menstrual flow in a short menstrual cycle may be helpful in the diagnosis of the disease.

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The lateral spread of clobetasol 17-propionate in the stratum corneum *in vivo*

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SUMMARY

Radiolabelled clobetasol 17-propionate in two different bases, propylene glycol (PG) and di-n-butyl adipate (BA), was applied to the backs of 10 normal male volunteers, and the application sites protected. After 8 h samples of stratum corneum (SC) were taken from the application site and an adjacent site, and the level of steroid measured. At the application site there were higher concentrations of the PG formulation at the deeper levels of the SC. At the adjacent sites there were higher concentrations of the BA formulation at the superficial levels. The mean radioactivity at the adjacent sites expressed as a percentage of that at the corresponding application sites (± SEM) was 7·8 ± 5·3% for the BA formulation and 3·0 ± 2·9% for the PG, ($P = 0{\cdot}0002$). Total percentage of the applied dose in the skin adjacent to the application site was 11·7 ± 6·3% for the BA formulation and 5·3 ± 5·7% for the PG ($P < 0{\cdot}0002$). Clobetasol 17-propionate, therefore, moves laterally to a greater extent in BA than in PG.

When a drug is applied to an area on the skin surface, its ability to penetrate directly through the stratum corneum (SC) is of prime importance in its effectiveness as a topically active agent. Much effort has been expended in devising methods of measuring drug flow across the SC, the assumption usually being made that flow only takes place perpendicularly through the SC. For practical purposes this may indeed approximate to the truth, but it is clear that if a drug can penetrate transversely across a certain thickness of SC it can also move laterally to a distance at least equivalent to the thickness of the SC and probably further. The main purpose of the present study was to try to measure lateral spread in the SC of a topically applied steroid, because if such spread does take place, its measurement would allow appropriate adjustment to be made to the interpretation of trans-SC drug flow in other studies. The second purpose of the study was to determine whether different bases made any difference to the lateral spread of a topical corticosteroid in the SC.

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METHODS

The basic concept of the method used was to apply a measured amount of radiolabelled steroid ointment to an area, protect the site, and then sample the adjoining SC after a fixed time period.

Ten normal male volunteers, over the age of 35 and with no history of skin disease, who were staff of the Southern General Hospital took part in the study. Written informed consent was obtained from all volunteers, and the study was approved by the local Ethical Committee.

Two ointment formulations of clobetasol 17-propionate were tested; one with a propylene glycol (PG) base and the other with di-n-butyl adipate (BA) as the base. The ointments contained tritium labelled clobetasol 17-propionate (12 MBq/g ointment). The ointments were supplied in tubes with small nozzles so that measured amounts could be delivered.

On the upper back of each subject six square test sites, 1·5 cm × 1·5 cm, were drawn in a symmetrical pattern with a ball-point pen. For each application, a strip of ointment approximately 3 cm long was applied to the outer curved end of a plastic test-tube, and this was weighed. The ointment was then spread evenly over the relevant square using the test-tube as the applicator and the test-tube then re-weighed.

The application site was protected by applying a small perspex box with one side open. The inner dimensions of the box were approximately 1·8 cm × 1·8 cm, so the edges of the box did not touch the SC to which the ointment had been applied. Three edges of the box were sealed to the SC with a small amount of rapidly setting cyanoacrylate glue (Powabond, Double H International Ltd, Staines, Middlesex, U.K.). The base of each box had holes in it so as to reduce any elevation of humidity within. Each box was further secured using several long strips of Micropore® adhesive tape.

This procedure was carried out on all six sites on each volunteer. PG based ointment was applied to three sites and BA based ointment to the rest, using a random allocation method.

After application, the volunteers carried out a normal day's work. Eight hours following application, the protective coverings were removed. Five successive skin surface biopsies[1] were taken from each of the six sites of application and from each of six immediately adjacent sites. The immediately adjacent sites had one lateral edge approximately 1 mm from the lateral edge of the marked site of application. The sites of the skin surface biopsies in relation to the sites of application are shown in Figure 1.

To measure the radioactivity in the SC biopsy samples, the portion of each glass slide with the attached skin surface biopsy was cut into two 2 cm × 1 cm sections using a diamond stylus and placed in a 20 ml glass scintillation vial (Canberra Packard, Pangbourne, Berkshire, U.K.). A 2 ml volume of a tissue solubilizer (Soluene-350, Canberra Packard), was poured into the vial over the sections of glass slide, the vial was capped, and then placed on its side to ensure complete immersion of the biopsy section in the solubilizer. After 2 h, 15 ml of a xylene-based scintillation fluid (Dimilume-30, Canberra Packard) was added to each vial. A minimum of 16 h later, the sections of glass slide were removed from each vial and discarded.

Diluted standards were prepared for each ointment by putting 0·1 g into pre-weighed scintillation vials and re-weighing; 10 ml of xylene was added to each vial and the vial shaken vigorously until the ointment had dissolved. A 100 μl volume of the resulting solution was then added to a second scintillation vial containing 2 ml Soluene-350 and 15 ml Dimilume-30 for counting.

Any radioactive ointment adhering to the perspex boxes used to cover the application sites was dissolved by immersing each box in 100 ml xylene and agitating gently for 10 min. A 1 ml

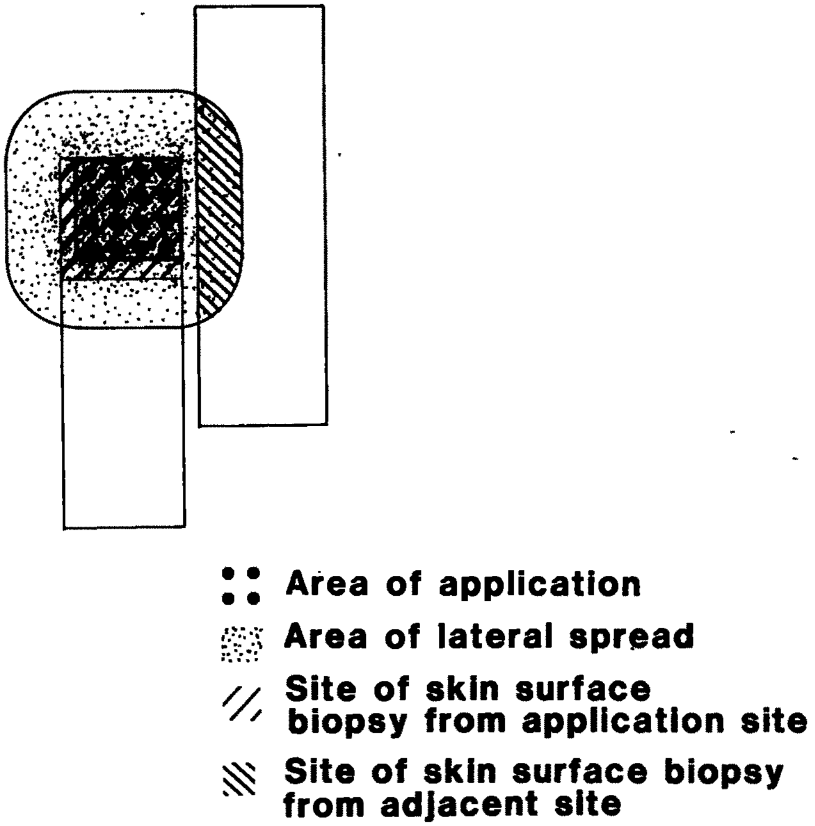
Area of application
Area of lateral spread
Site of skin surface biopsy from application site
Site of skin surface biopsy from adjacent site

FIGURE 1. Diagram to show the sites of the skin surface biopsies in relation to the site of application of steroid.

aliquot of the resulting solution was then added to a scintillation vial containing 2 ml Soluene-350 and 15 ml Dimilume-30.

Radioactivity was counted in a liquid scintillation counter (Packard Tricarb A4430) with external standard correction for differing counting efficiencies. The radioactivity in each biopsy or perspex box was then expressed as a percentage of the dose applied, the latter being calculated from the radioactivity in the appropriate diluted standard.

In two additional volunteers, rigorous occlusion was applied to the adjacent sites to ensure prevention of lateral smearing, and the results in these individuals were compared with those in the other subjects.

Statistical analysis

The non-parametric Mann-Whitney U test was used for statistical analysis.

RESULTS

Each strip of ointment weighed approximately 0·012 g. Therefore the radioactive label applied was approximately 0·9 MBq per volunteer.

In two of the 10 subjects, two of the covers became dislodged during the study so

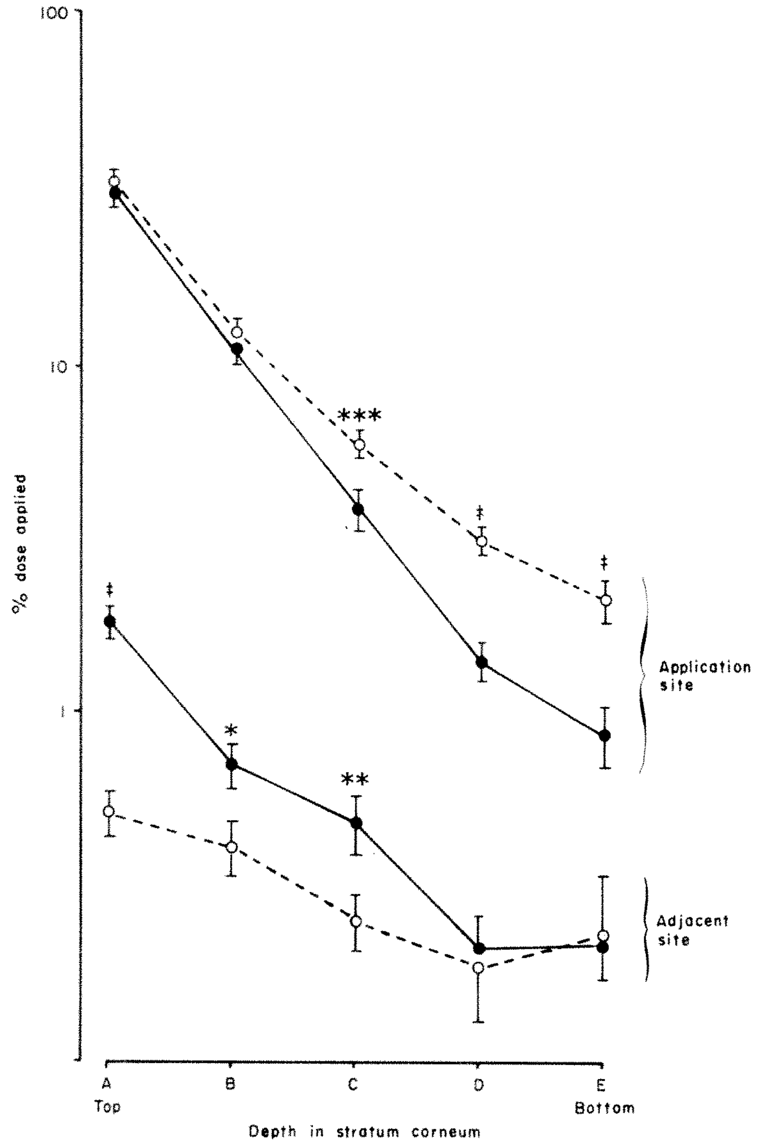

FIGURE 2. Levels of radiolabelled steroid detected at the application site ($n=28$) and at the adjacent site ($n=28$) for clobetasol 17-propionate, in PG (○) and BA (●) at each level of the SC, where A is the outermost level and E the deepest level sampled. Points are means ± SEM ($n=28$). PG value significantly different from BA * $P<0{\cdot}01$; ** $P<0{\cdot}005$; *** $P<0{\cdot}001$; ‡ $P<0{\cdot}0002$.

measurements could not be taken at these sites. Thus, a total of 28 sites was assessed for each ointment.

The results for the 10 subjects show that at both application and adjacent sites the radioactivity levels for each ointment decreased approximately exponentially with SC depth (Fig. 2). At the application site, the radioactivity levels for the PG and BA ointments were similar in the two outermost SC layers (A and B) but in the inner layers (C, D and E) the levels of

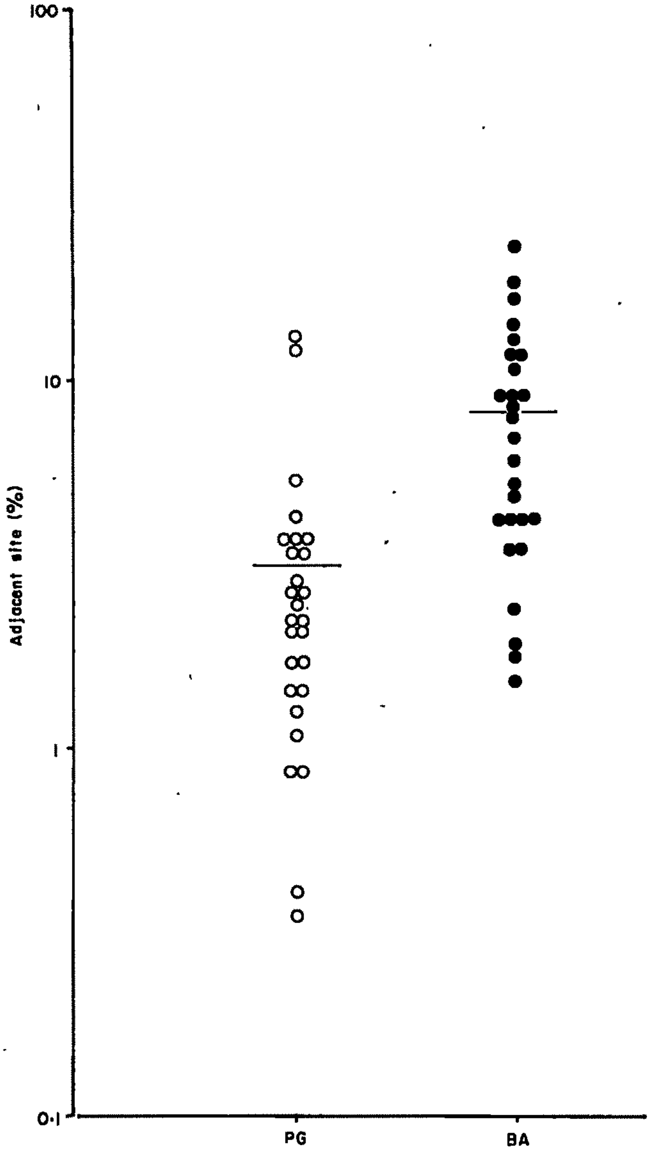

FIGURE 3. The total radioactivity detected at each adjacent site expressed as a percentage of the total radioactivity at the corresponding application site. Horizontal lines indicate means. BA value significantly greater than PG, $P < 0{\cdot}0002$.

the PG ointment were significantly higher. When the total radioactivity levels in the SC at the application site were calculated (i.e. sum of activities in all five layers), there was no significant difference between the PG and BA ointments (mean ± SEM 58·4 ± 14·8% of applied dose and 51·0 ± 18·4%, respectively), because the total radioactivity was largely made up of radioactivity in the outer layers.

The mean radioactivity levels at the adjacent sites were generally less than 10% of the corresponding application site values. The BA formulation gave significantly higher values than

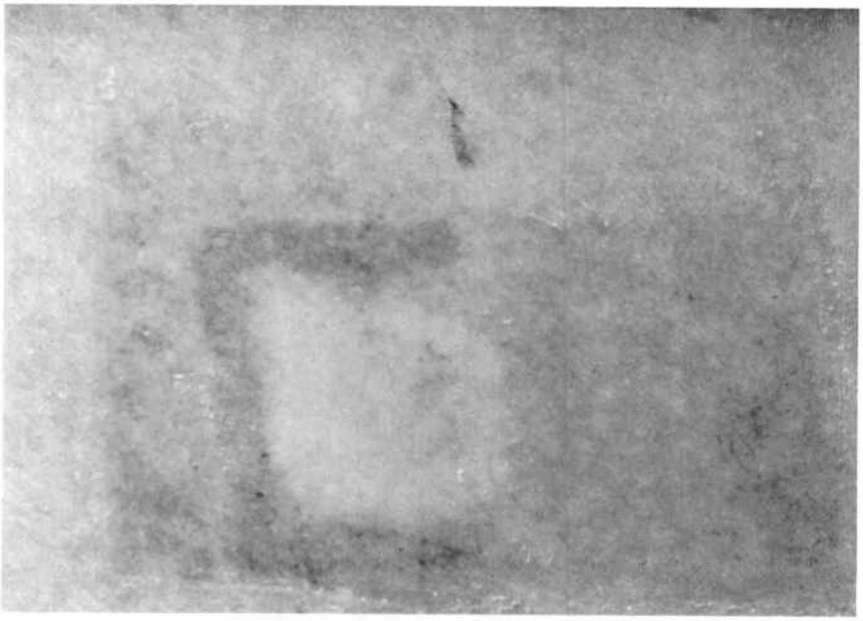

FIGURE 4. Site of application (pale area) and adjacent sample site to the right. A well defined localized pallor can be seen at the site of application.

the PG in the outermost SC layers (A, B and C) while there was no significant difference in the two innermost layers (D and E). When the individual biopsy layer values were summed to give the total radioactivity levels in the SC at the adjacent sites, the mean value for the BA formulation was significantly higher than that for the PG (3·5 ± 1·9% and 1·6 ± 1·7%, respectively; $P < 0{\cdot}0002$). When the radioactivity at each adjacent site was expressed as a percentage of that at the corresponding application site, the mean value for the BA formulation was significantly higher than that for the PG (7·8 ± 5·3% of applied dose and 3·0 ± 2·9%, respectively; $P = 0{\cdot}0002$) (Fig. 3). It can be shown geometrically that the adjacent biopsy site accounts for approximately 30% of the area outside the application site into which the ointments may migrate, therefore an estimate of the total radioactivity outside the application site could be made. When this was done, the mean percentage of the applied dose in the surrounding skin adjacent to the application site was calculated as 11·7 ± 6·3% for the BA formulation and 5·3 ± 5·7% for the PG ($P < 0{\cdot}0002$).

In six of the subjects (a total of 16 sites for each ointment), the protective perspex boxes were assayed for radioactivity. Radioactivity ranged from 0·2 to 72·2% of the applied dose. However, there was no significant difference between the two ointments with respect to residual radioactivity on the boxes (23 ± 21·6% of applied dose for the BA ointment and 11·6 ± 13·9% of applied dose for the PG).

When the radioactivity on the perspex boxes was added to the total radioactivities at both the application site and the adjacent site, and subtracted from 100%, we were left with the radioactivity which diffused inward beyond the SC. In the six subjects for whom this could be calculated, the mean percentage inward diffusion beyond the SC was 20·6 ± 19·3% of applied dose for the BA ointment and 29·3 ± 20·2% of applied dose for the PG. These results were not significantly different.

The results from the two volunteers in whom extra efforts were made to prevent contamination showed a pattern which was essentially similar to that seen in the other subjects, confirming that superficial smearing of topically applied steroid was not responsible for our observations.

DISCUSSION

The results obtained in the present study suggest that while the two ointments behave similarly in terms of skin penetration, the BA ointment spreads laterally to a greater extent than the PG ointment, so that levels of BA tend to be lower at the application site and higher at the adjacent site. This lateral spread was significant for both ointments in that 5–12% had spread out from the application site after 8 h. From measurement of the total radioactivity recovered from the skin and the perspex boxes, we estimated that 20–30% had diffused down through the stratum corneum to the epidermis. Therefore, the amount of ointment diffusing laterally is important and cannot be ignored when assessing the kinetics of ointment moving through the stratum corneum, especially when the area of ointment application is small, as in the present study.

The residual radioactivity on the perspex boxes was very variable. The high values were almost certainly due to contamination of the inner surface of the box as the skin at the application site flexed and bulged within the box which was only 0·3 cm deep. This transfer of ointment to the box would affect the radioactivity at the application site and the adjacent site as the ointment would be unavailable to the skin. However, the ratio of radioactivity on adjacent site to that on the application site would be much less affected.

The major practical difficulty that this study posed was to ensure that there was no superficial contamination of the adjacent sites from the sites of steroid application. In a normal clinical situation there would be rapid and extensive contamination of the dressing, clothes and wide areas of the skin surface. Great care was taken to avoid such contamination. The occlusion with the perspex boxes was designed to overcome this problem and where the boxes had obviously slipped the data were excluded. In reality there may have been some occasional minor slippage of the boxes and it is impossible to be certain or to prove that no superficial contamination took place. However, given that the ointments were randomly allocated to sites over the back throughout the study, the fact that a significant difference was found between the activities of the ointments on the adjacent sites implied that the results obtained were valid and not artefactual.

At the adjacent sites, the superficial levels of the SC usually contained the largest concentration of radiolabelled steroid. This might support the view that there was superficial contamination. However, even if there was absolutely no contamination, there would still be a gradient across the SC with a lower concentration adjacent to the 'sink' side of the underlying epidermis. We feel that the results of the additional experiment provide further confirmation that true intracorneal lateral spread does take place.

The formulation of clobetasol propionate in the low viscosity oil di-n-butyl adipate in wax and liquid paraffin should result in partitioning of the steroid more favourably into the oil, encouraging greater spread within the SC.[2] The results of our study suggest that there is indeed greater lateral spread of the steroid in the BA base than in the PG base. These findings are also compatible with our previous investigation of the SC penetration characteristics of clobetasol 17-propionate in these two bases.[3]

Lateral spread of topical cypermethrin within the SC of sheep has been reported.[4] This finding together with our own results suggest that the amount of lateral spread is surprisingly large.

The detection of lateral spread of topical steroid in the SC in humans *in vivo* is of interest, but its functional significance remains obscure. When a potent topical steroid is applied to a well defined area of skin, the pallor induced remains confined to the area of application and is not seen to spread. We observed this well localised pallor in the present study (Fig. 4). It is possible, therefore, that while the main flow of topical steroid takes place directly through the SC at the site of application, the lateral spread within the SC may remain largely confined to the SC and may have little functional significance. The occurrence, however, of this phenomenon does need to be recognized when measuring the flow of drugs within the SC.

ACKNOWLEDGMENTS

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An evaluation of itraconazole in the management of onychomycosis

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SUMMARY

At present the reported use of itraconazole, a new oral triazole antifungal, has been confined to short-term treatments. This investigation is an appraisal of itraconazole in the treatment of three different forms of onychomycosis. Six patients with nail infections due to *Candida albicans* not associated with paronychia affecting a total of 20 nails received itraconazole (100 mg daily) for a mean period of 5·9 months. Complete remission was achieved in all cases. Twenty six patients with dermatophyte onychomycosis affecting a total of 45 finger and 80 toe-nails were treated with itraconazole (100–200 mg daily). In 24 cases the causative organism was *Trichophyton rubrum*. Remission was achieved in 64% of finger and 73% of toe-nails in 5 and 9·4 months, respectively. Treatment failures were experienced in patients with finger-nail infections due to *T. violaceum* (1) and those concurrently receiving phenytoin and phenobarbitone (3). Three patients with infections due to *Hendersonula toruloidea* failed to respond to treatment. Adverse effects were experienced by four patients (abdominal discomfort 3, diarrhoea 1), but none were serious enough to lead to abandonment of treatment. No persistent changes were seen in serum biochemical values. This study suggests that itraconazole is potentially effective in the long-term treatment of superficial fungal infections such as onychomycosis, and comparative studies with alternatives such as griseofulvin should now be carried out.

Fungal infections of the nails, onychomycoses, are notoriously difficult to treat, the main problems being the length of therapy required and the poor overall response rate. Very few topically applied drugs have shown benefit in the management of these infections, and treatment rests with the use of oral antifungals. Even so, there are some infections, such as those caused by *Scopulariopsis brevicaulis*, which do not respond to any of the existing systemic antifungals,[1] a clinical observation which is supported by a corresponding lack of *in vitro* activity.

For nail infections caused by dermatophytes, griseofulvin remains the treatment most commonly used. However, it has been shown that the remission rates at follow up after prolonged therapy for 1 year may be as low as 40%, the poorest responses being seen with toe

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nail infections.[2] Oral ketoconazole is an alternative drug, but long-term studies have shown a similar low response rate in toe nail infections[3] and the risk of hepatitis, although rare, makes it a drug of second choice in this form of nail disease. Candida onychomycosis, however, responds well to ketoconazole.[4] For other types of onychomycosis, such as nail infections due to *Hendersonula toruloidea*, there are no consistently effective forms of treatment.

Itraconazole is a new oral triazole antifungal drug.[5] *In vitro* it is highly active against dermatophytes as well as *Candida* and some *Aspergillus* species. Early clinical studies have shown that it is active against dermatophyte infections, with good responses being seen despite apparently low serum levels. This may be accounted for by strong binding to tissues including stratum corneum.[6] The purpose of the present work was to extend our previous studies of the efficacy of itraconazole in superficial fungal infections[7] to the management of nail disease.

METHODS

All patients were attending St John's Hospital for Diseases of the Skin. The diagnosis of onychomycosis was confirmed by direct microscopy of nail clippings and by culture on Sabouraud's medium with and without cycloheximide.

Three groups of patients were studied: (1) Six patients had *Candida* onychomycosis without paronychia. They were diagnosed by the presence of characteristic nail dystrophy,[8] the demonstration of both yeasts and hyphae in nail clippings, and the isolation of *Candida albicans* in culture. None had chronic mucocutaneous candidosis. Five had peripheral vascular disease, in all cases Raynaud's disease; the other patient had been treated with oral prednisolone for asthma in doses of up to 40 mg daily. She had clinical features of Cushing's syndrome including bruising, obesity and hypertension. There were four females and two males, mean age 61 years (range 52–71 years). (2) Twenty-six patients had onychomycosis due to dermatophytes and were either unresponsive to griseofulvin given in doses of at least 10 mg/kg daily for a minimum period of 9 months for finger-nails and 1 year for toe-nails, or had relapsed within 3 months of stopping the drug. Two patients were unable to tolerate griseofulvin. Predisposing abnormalities were found in seven patients, namely diabetes mellitus (1) peripheral vascular disease (4) tylosis (1) and polycythaemia rubra (1). In 24 individuals the infection was caused by *Trichophyton rubrum*, in one by *T. interdigitale* and one by *T. violaceum*. There were 19 males and seven females and a total of 125 nails were infected. The mean age of the patients was 47 years (range 28–73 years). (3) Four patients had *Hendersonula toruloidea* infections of the finger nails. All had originated from the West Indies up to 14 years before presenting for treatment. There were three males and one female, mean age 48·5 years (range 36–69 years).

All the patients entering the study had not received oral or topical antifungal therapy for at least 6 weeks before starting itraconazole. The initial dose was 100 mg daily and this was increased to 200 mg daily if there was no significant progress within 2 months of starting on the treatment. In all cases patients were seen after 2 weeks, 1 month and monthly thereafter throughout the study. The mycological investigations were repeated at each visit. Blood samples were taken at each visit for full blood count, liver function tests and plasma electrolytes. Patients were also seen 1, 3 and 6 months after the end of therapy.

Assessment of nail disease

Nails were assessed as cured if there was complete clinical improvement and negative microscopy and cultures at the end of treatment. In 10 patients with dermatophytosis of the toe-nails, the rate of response was assessed for the worst affected nail by tracing the outline of the infected portion of nail before treatment (time 0) and at 3, 6, 9 and 12 months, using a method described previously.[9] The rate of improvement of the nails was calculated according to the following formula:

$$\frac{\text{Area 'normal' nail (time } x) - \text{Area 'normal' nail (time 0)}}{\text{Area 'normal' nail (12 months)} - \text{Area 'normal' nail (time 0)}} \times 100 = \%\text{ regrowth.}$$

Minimum inhibitory concentrations

Minimum inhibitory concentrations (MIC) of itraconazole were measured in yeast nitrogen broth as described previously.[12]

RESULTS

Candida onychomycosis

All six patients had finger nail dystrophy and the total number of nails infected was 20. They all responded to itraconazole given in a dose of 100 mg daily for a mean period of 5·9 months (range 3–8 months). At the end of treatment all had attained clinical and mycological remission (Figs 1a and b). In the post-treatment period of observation one patient had relapsed at 5 months, but responded to a further course of 3 months of therapy. She remained well at the time of writing.

Onychomycosis due to dermatophytes

A total of 26 patients were treated for 1 year or until recovery, whichever was the shorter period. The dose of itraconazole was increased to 200 mg daily after 2 months in 5 patients.

The nail infections could be divided into a total of 45 affected finger-nails and 80 toe-nails. At

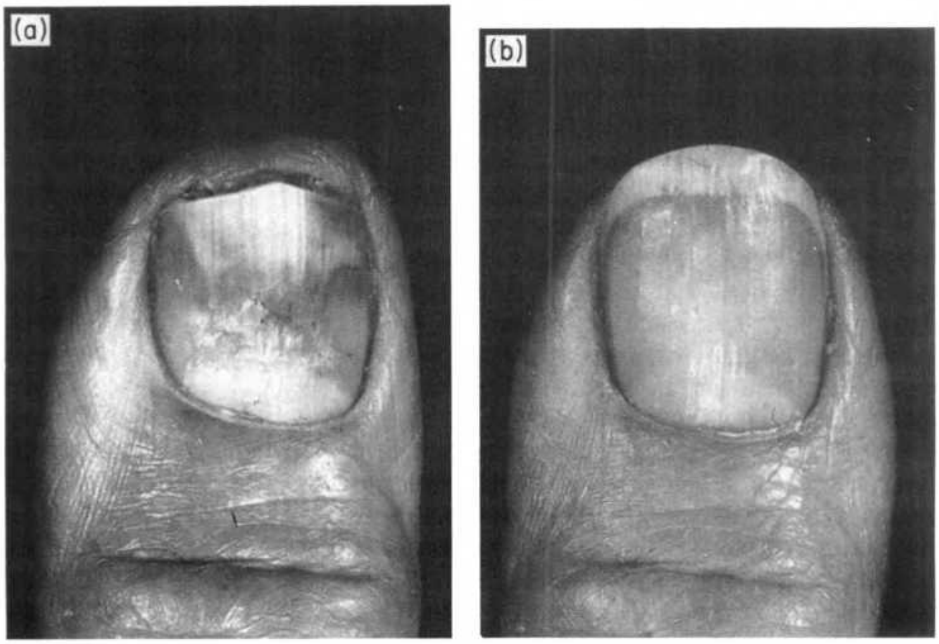

FIGURE 1. (a) Onychomycosis due to *C. albicans* in a patient with Raynaud's disease, before treatment. (b) Same nail after 5 months on itraconazole.

the end of therapy the clinical and mycological remission rates were 29 (64%) for finger nails and 58 (73%) for toe-nails. The mean periods of therapy were 5 months (range 3–7 months) and 9·4 months (range 5–12 months) respectively. The patient with a *T. interdigitale* infection of one finger cleared after 5 months therapy but relapsed after a further 6 months. In contrast, the patient with an infection due to *T. violaceum* failed to show any response after 4 months and treatment was stopped. One patient with a *T. rubrum* infection of three toe-nails abandoned treatment after 2 months and was not included in the assessment. The remaining 23 cases were assessed further and the results, expressed as cumulative clinical cures, are shown in Figure 2. It can be seen that the cumulative remission rates at the end of treatment for *T. rubrum* infections were 80% for finger-nails and 76% for toe-nails. These figures include three patients who had been receiving long-term therapy with phenobarbitone and phenytoin for epilepsy. In two of these the serum levels of itraconazole were low (> 25 and 45 ng/ml, respectively), when assayed by high pressure liquid chromatography. The normal range is 200–500 ng/ml. If these patients are excluded the total percentages of nails achieving remission were 100% for finger-nails and 76% for toe-nails (Figs 3a and b).

The rate of response of the worst affected toe-nail in 10 patients is shown in Table 1. Four (14%) finger-nails and nine (16%) toe-nails cured at the end of treatment showed clinical and mycological relapse 6 months later.

Onychomycosis due to Hendersonula toruloidea

Four patients with a total of 17 affected nails were treated. After 4 months, during which the dose was increased to 200 mg itraconazole daily, there was no evidence of improvement in any patient and treatment was stopped.

Minimum inhibitory concentrations

The clinical findings generally correlated with the susceptibility of the fungi to itraconazole *in vitro*. All the *T. rubrum* isolates tested were sensitive (MIC range < 0·25–4·0 mg/l) as were those of *C. albicans* (MIC range < 0·25–2·5 mg/l). In contrast, all isolates of *H. toruloidea* examined grew at concentrations of 64 mg/l. The isolates of *T. interdigitale* and *T. violaceum* were both susceptible to 0·25 mg/l itraconazole.

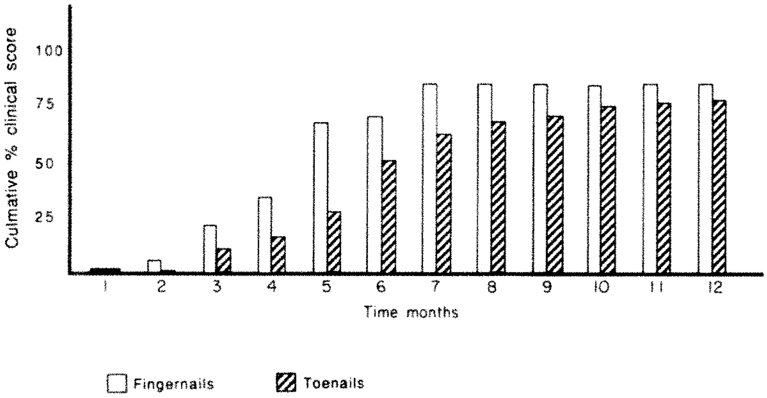

FIGURE 2. Cumulative clinical and mycological cure rates in 23 patients with onychomycosis due to *T. rubrum*.

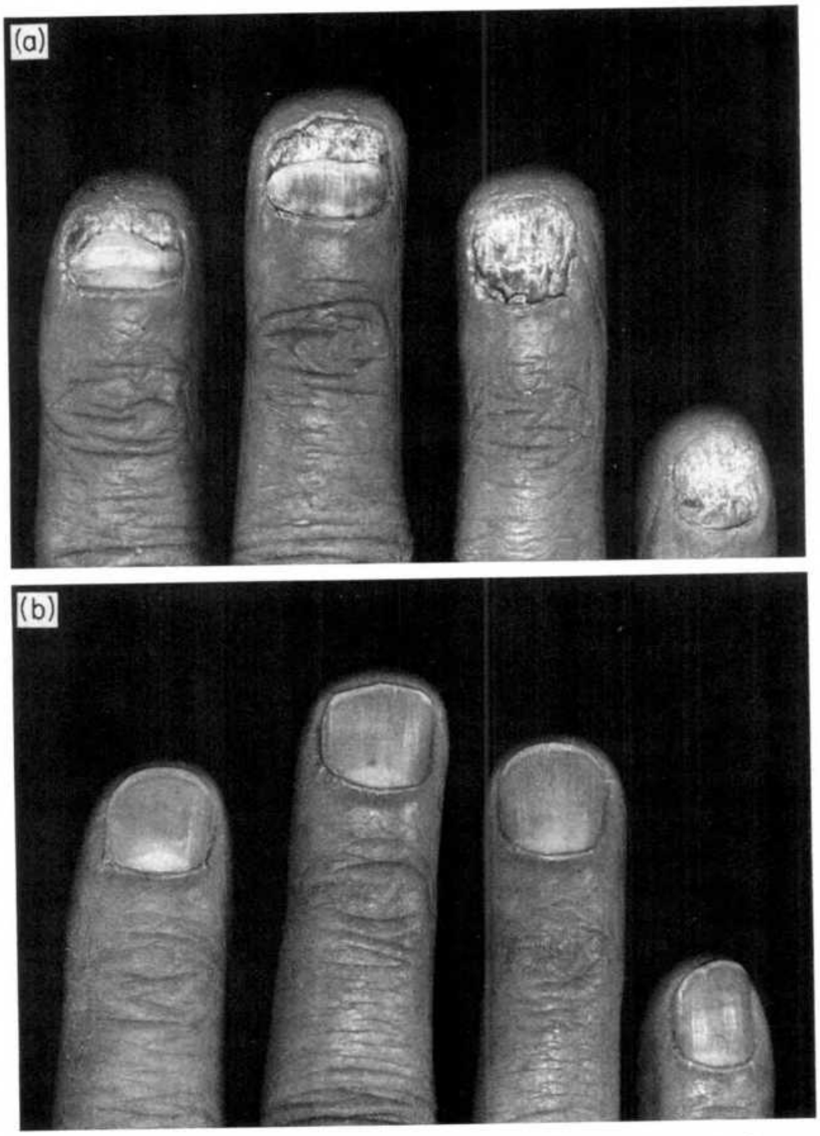

FIGURE 3. (a) Onychomycosis due to *T. rubrum*, before treatment. (b) Same finger nails after 7 months on itraconazole.

TABLE 1. Percentage regrowth of worst affected toe nails in 10 patients during itraconazole therapy. Values are means ± SD

Time (months)			
3	6	9	12
31 ± 10	61 ± 16	86 ± 14	100 ± 15

Adverse effects

No patient stopped treatment because of adverse effects. Three patients recorded abdominal discomfort and nausea in the first 2 weeks of therapy, which resolved without stopping the drug. One patient had diarrhoea and temporarily interrupted therapy until the episode had resolved which took 5 days; but this was thought to be unrelated to the drug. No patient reported headache. There were also no abnormal laboratory results during the treatment, apart from one patient whose alkaline phosphatase levels rose from 11 to 32 U/ml, but which returned to normal (< 18 U/ml) after a further month, without interrupting therapy.

Three patients taking itraconazole were on long-term anti-epileptic treatment with phenobarbitone and phenytoin. All three had failed to respond to griseofulvin and one had also been given ketoconazole without success. The low serum levels of itraconazole, which we found in two of these patients, suggest that the concurrent administration of the anti-epileptics had interfered with the metabolism of itraconazole.

DISCUSSION

Previous studies have shown that itraconazole is effective in both dermatophyte and superficial *Candida* infections. The most appropriate dose for dermatophytosis appears to be 100 mg daily, with treatment regimens using 50 mg doses being less successful. Saul *et al*,[10] for instance, recorded only 55% remission rates for dermatophytosis after 60 days using the 50 mg dose. Remission rates of 87% and 83% have been recorded respectively for tinea corporis or cruris and tinea pedis using the 100 mg dose regimen.[11] In chronic infections it has proved possible to induce remissions in patients with persistent infections due to *T. rubrum* unresponsive to griseofulvin after mean treatment times of 6·2 weeks for tinea corporis and 8·0 weeks for dry type hand infections.[12] In these latter studies the effect of the drug on nail disease was not recorded. Likewise, itraconazole works well against vaginal candidosis[13] and chronic oral candidosis secondary to chronic mucocutaneous candidosis (CMCC).[12] It has also been used successfully to treat a patient with a widespread cutaneous and mucosal candida infection associated with CMCC.[14]

The present study shows that itraconazole has a role to play in the longer term treatment of dermatophytosis affecting the nails. All the patients treated had either failed to respond previously to griseofulvin or, in two cases, been unable to take the drug because of adverse effects, usually headache or nausea. The reasons for failure of griseofulvin therapy are clearly complex.[15] In some cases lack of absorption or penetration of the drug have been cited as an explanation. In others the presence of underlying disease, such as tylosis, or failure of immune responses have been factors. In the present study three patients on long-term anti-epileptic treatment with phenobarbitone and phenytoin, all previous treatment failures on griseofulvin, also failed to respond to itraconazole. In two cases it was found that serum levels of the drug were suboptimal suggesting that induction of metabolic liver enzymes by these drugs had facilitated breakdown of the itraconazole. Patients on griseofulvin[16] or ketoconazole[17] are also unable to sustain adequate serum levels of the antifungal while on treatment with these anti-epileptics.

In order to confirm these results in dermatophytosis it would be necessary to compare itraconazole with griseofulvin in a controlled long-term study. It would also be important to know whether treatment with shorter courses of itraconazole can improve or cure nail disease as the need to take medication for a period of a year, or longer, in toe nail infections is a considerable disincentive to many patients and improvements in this area would clearly be useful. This study also demonstrated the effectiveness of itraconazole in the treatment of *Candida* onychomycosis and this drug may, therefore, prove to be a useful alternative to ketoconazole in this infection. Itraconazole produced no improvement in nails infected by *H. toruloidea*. Thus this condition has once again been found refractory to oral therapy.

During the present study no patients recorded any adverse effects which led to interruption of the treatment. Those recording ill effects included three patients with abdominal pain and nausea. It is possible that the recent introduction of a new formulation of oral itraconazole may reduce the incidence of this side-effect, although numbers reporting gastrointestinal discomfort were few. No persistent changes in biochemical parameters, including liver function tests, were recorded. While this does not exclude rare idiosyncratic hepatic reactions, these early results are clearly encouraging as they suggest that itraconazole may prove to be an effective alternative to griseofulvin for long-term treatments, provided that subsequent experience confirms the lack of toxicity.

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Diphencyprone in the treatment of long-standing alopecia areata

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SUMMARY

Thirty-six patients with alopecia areata of 1–54 years duration entered a study of treatment with the contact allergen diphencyprone for 8 months. Following sensitization the diphencyprone was applied to one half of the scalp at weekly intervals, the other half acting as a control. Once hair growth was established on one side, the other side was treated. Seven patients did not continue treatment and one patient showed spontaneous regrowth. Of the remaining 28 patients who persisted with treatments, fourteen (50%) regrew hair on the treated side; eight (29%) had a cosmetically acceptable result with the regrowth of terminal hair over the whole scalp. No statistically significant differences were found in age or duration of alopecia between those who regrew and those who did not. We have found diphencyprone to be an effective stimulator of hair growth in patients with severe and long-standing alopecia areata.

Alopecia areata affects 2% of patients presenting to dermatological outpatient departments in Britain and the U.S.A.[1] Treatment, however, is still diverse and includes the application of irritants such as dithranol and croton oil, the use of ultraviolet radiation in the form of PUVA or UVB alone, topical or systemic steroids, minoxidil, and the application of contact allergens. Results from these treatments have been varied and unpredictable, but considerable success has been achieved with contact allergens applied to the scalp; these include dinitrochlorobenzene (DNCB)[2], squaric acid dibutyl ester (SADBE)[3] and diphencyprone (2,3 diphenylcylopropenone-1) (DPC).[4] Dinitrochlorobenzene has recently been shown to be mutagenic in the Ames test and is, therefore, considered unsuitable as a treatment for alopecia areata.[5,6] Squaric acid dibutyl ester is non-mutagenic in the Ames test, but is very unstable. Diphencyprone, however, is both non-mutagenic in the Ames test[4,7] and non-teratogenic in chick and mouse embryos (R. Happle, personal communication). It is a potent contact sensitizer which can be obtained as a 98% pure powder, and which in solution is stable for 4 weeks if kept at room temperature and protected from ultraviolet radiation.[8] It is not known to cross-sensitize. Diphencyprone has been used by Happle and by other workers since 1982 in the treatment of alopecia areata, with success rates of between 65% and 70%. These authors, however, do not mention the duration of

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the alopecia. The present study was, therefore, designed to investigate the effectiveness of DPC in patients with long-standing alopecia areata.

METHODS

The criterion for entry to the study was extensive alopecia areata of at least 1 year's duration. Ethical Committee approval was obtained, and each patient gave informed consent.

Thirty-six patients (11 men, 25 women, aged 18–58 years) were studied. The duration of alopecia ranged from 1 to 54 years (mean 19·9 years). Twenty-nine patients had been treated unsuccessfully in the past with one or more of the accepted alternative forms of treatment, UVB, dithranol, minoxidil, topical steroids and, in two cases, DNCB.

Diphencyprone was dissolved in acetone and stored at room temperature in amber glass bottles. It was applied using a cotton wool bud on the end of a 6″ orange stick. Initially sensitization was achieved by painting one-half of the scalp; the other half acting as a control. Subsequently, the area of the scalp used for sensitization was reduced to avoid eliciting extensive allergic reactions which might involve the rest of the scalp and face. At the first visit, a 2% solution was applied to induce sensitization and the patient was seen 2 weeks later to determine whether sensitization had developed. Treatments were performed weekly thereafter. The concentration was adjusted individually depending on the severity of the previous reaction and varied between 0·0001% and 2%. The aim was to maintain erythema and pruritus on the treated side for 24 to 36 h after application. Once hair growth was established on one side the other side was treated.

Whenever possible, patients were treated on a Friday afternoon so that any irritant reaction had settled by the following Monday. They were also supplied with a small amount of a potent steroid cream for 'first-aid' use in the event of too vigorous a reaction.

Measurements of full blood count, electrolytes, and renal and hepatic function were made monthly. Thyroid function tests were carried out in 24 patients. In 28 patients the levels of anti-adrenal cortex, anti-smooth muscle cell, anti-gastric pariatal cell, anti-reticulin, anti-mitochondrial, anti-nuclear, anti-thyroid microsomal and anti-thyroglobulin antibodies were measured. The presence of rheumatoid factor and the levels of immunoglobulins, IgG, IgM, IgA, IgE, and complement C_3 and C_4 were also estimated in these 28 patients.

Initial attempts at grading the alopecia showed that the patterns of hair loss did not fit easily into the accepted definitions of alopecia areata, totalis and universalis. For example, many patients had a predominantly bald head, but with a scattering of terminal hairs not arranged in tufts or patches; they were not bald enough to be classified as alopecia totalis, but were more severely affected than the average patient with alopecia areata. Equally, there were patients who had a completely bald scalp with partial body hair loss; they had too much hair to be described as alopecia universalis, but the loss of some body hair excluded them from the classification of alopecia totalis. Therefore, a simple classification was devised which described scalp and body hair separately (Table 1). Thus, grades 1A and 2A would indicate alopecia areata of increasing severity, whilst grades 4A and 4C would refer to alopecia totalis and alopecia universalis, respectively.

The end-point of therapy was hair growth. We observed several different types of regrowth which were graded as follows: grade 1, vellus hair; grade 2, sparse pigmented terminal hair; grade 3, terminal growth with patches of alopecia and grade 4, growth of terminal hair over all of the scalp.

TABLE 1. Grading of alopecia areata prior to treatment

	Grade
Scalp	
Mild to moderate alopecia areata; < 50% of the scalp involved	1
Severe alopecia areata > 50% of the scalp involved	2
Sub-total alopecia with scattered terminal hairs present only	3
Total alopecia	4
Body	
No loss	A
Partial loss	B
Total loss	C

Statistical analysis

The age, duration of alopecia and the number of applications required to sensitize those patients who regrew and those who did not were compared using the Wilcoxon signed ranks test.

RESULTS

Thirty-six patients started treatment; 29 (22 women, seven men; age range 18–56 years) continued treatment for 8 months or longer. Seven patients discontinued treatment before adequate assessment could be made, five of these were unable to keep the weekly appointments and two could not tolerate a vesicular eczema which developed in the area of sensitization. One patient, after 8 weeks treatment to one side, showed bilateral regrowth which was considered to be spontaneous and he was withdrawn from the study. The results presented here are based upon the remaining 28 patients who continued treatment for 8 months. The initial grades of alopecia are shown in Figure 1. Of the 28 patients 22 (81%) required a wig, and four (males) preferred baldness to wearing a wig. In 14 patients (12 women, two men) hair regrew on the treated side and 14 showed no regrowth. Of these 14 patients eight (seven women, one man) showed regrowth of terminal hair over all the scalp—grade 4 in our classification (Fig. 2). Two showed grade 3 regrowth (terminal hair but with patches of alopecia persisting), 2 showed grade 2 regrowth (sparse terminal hair) and 2 regrew vellus hair only (Table 2).

One or more side-effects were experienced by most patients, but were self-limiting within 48–72 h. Blistering eruptions on the treated side of the scalp were seen in 12 of the 28 patients, periorbital oedema in four and eczematous rashes on other parts of the body, most notably the hands and forearms were seen in seven. Cervical lymphadenopathy occurred in all patients who responded to the contact allergen, and apart from initial tenderness did not cause the patients further discomfort.

Sensitization was achieved with the first application of 2% diphencyprone in 14 of the 28 patients (50%). Two patients failed to become sensitized. The mean number of applications required to sensitize the patients who regrew (1·4; range 1–4) was not significantly different from that in the patients who did not (2·2; range 2–6). At the time of writing, neither of the authors had become sensitized. There was no statistically significant difference between the ages of those who regrew (mean 39·7 years, range 18–56 years) and those that did not (mean 38·8 years, range 19–58 years), or in the duration of alopecia, mean 21 years (range 5–38 years) in

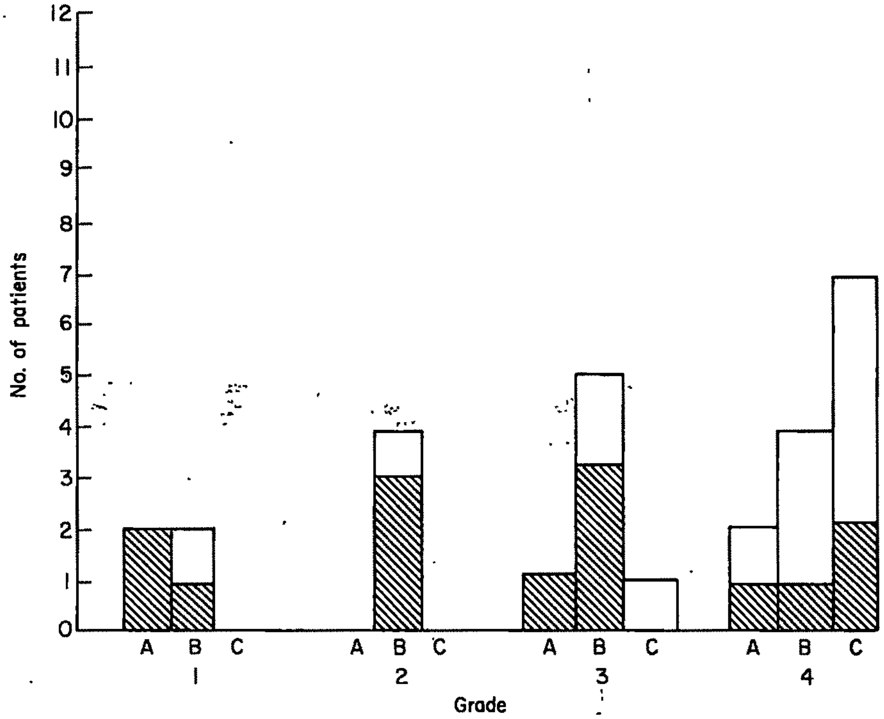

FIGURE 1. Pre-treatment alopecia grades in patients who responsed to treatment ■ and those who did not □.

those who regrew and 23·9 years (range 1–54 years) in those that did not. The time taken to regrowth varied between 5 weeks and 18 weeks (median 9·5 weeks). Those taking the longest time to start regrowth were both males, one with grade 2B alopecia (16 weeks) and the other with grade 4C alopecia (18 weeks).

Thirteen of the 28 patients (46%) had auto-antibodies (Table 3). Five patients had two auto-antibodies and one had three. However, the presence of auto-antibodies did not correlate with the patients' response to treatment. Of the 14 patients who regrew hair, nine had auto-

TABLE 2. Hair growth after 8 months of treatment with diphencyprone

	No. of patients
Total treated	28
No regrowth	14
Vellus hair	2
Sparse terminal hair	2
Terminal hair with patchy alopecia	2
Terminal hair over all the head	8

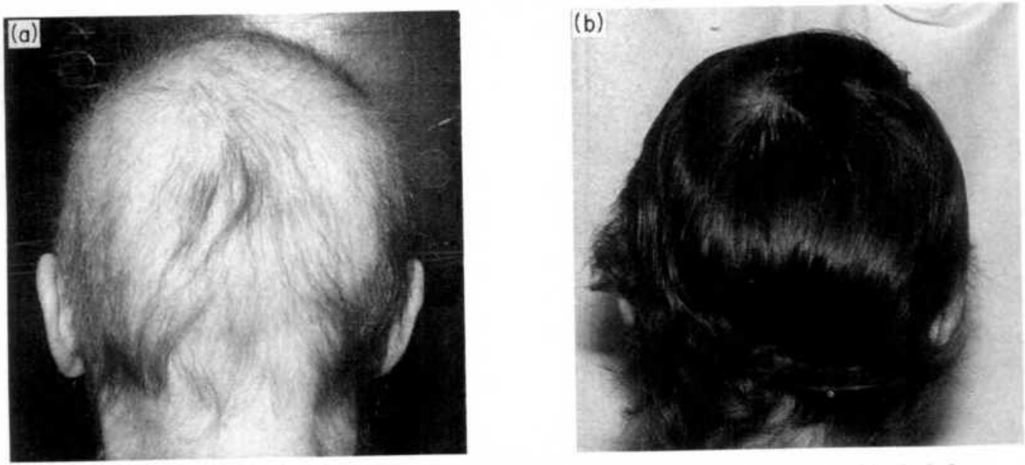

FIGURE 2. Twenty-nine-year-old woman with sub-total alopecia and partial loss of body hair (grade 3B) of 8 years duration (a) before and (b) after 8 months treatment with diphencyprone.

antibodies and three had more than one auto-antibody. Of the 14 who did not regrow, four had auto-antibodies and two had more than one auto-antibody.

Two patients were found to be biochemically hypothyroid. One patient had a high titre of anti-thyroid microsomal antibody and the other a low titre of this antibody; neither had anti-thyroglobulin antibody. Thyroid function tests were normal in the remaining patients.

One abnormal parameter, previously recognized by Safai *et al.*,[10] was the elevation of IgM levels, which was seen in 22 of the 28 patients. Values ranged from 144 to 610 IU/ml (normal 60–129 IU/ml) the mean being 249 IU/ml. In 10 patients the level was above 200 IU/ml. The other immunoglobulin levels were within the normal range. Elevated C_3 levels were seen in 10 of the 28 patients, values ranging from 162 to 198 IU/ml (normal 104–161 IU/ml), eight of these patients also had a raised IgM level.

TABLE 3. Auto-antibodies in patients with alopecia areata

	Patients who showed regrowth Patient no.									Patients who did not show regrowth Patient no.			
Antibody	1	2	3	4	5	6	7	8	9	1	2	3	4
Thyroid microsomal													
High titre $1:160^2$–$1:2560^2$	–	–	–	–	–	–	–	–	–	+	–	+	–
Low titre $1:10^2$–$1:80^2$	+	–	+	–	–	+	+	+	–	–	+	–	+
Thyroglobulin	–	–	–	–	–	–	–	–	–	–	+	–	–
Anti-nuclear factor	+ (1:10)	+ (1:50)	–	+ (1:10)	–	–	–	–	–	–	+ (1:50)	+ (1:10)	–
Gastric parietal cell	–	–	+	–	–	–	–	+	–	–	–	–	–
Smooth muscle cell	–	–	–	–	+	–	–	–	+	–	–	–	–

Once bilateral hair growth was established the frequency of the applications was successfully reduced to fortnightly or three-weekly in four out of five patients. However, one patient quickly began to lose hair over the crown; further loss was arrested by reverting to weekly treatments.

Haemoglobin, and white cell and platelet counts did not alter significantly over the 8 months treatment. Similarly renal and hepatic function remained unaltered.

DISCUSSION

The aetiology of alopecia is not fully understood; it is generally considered to be an auto-immune disease. Evidence for this hypothesis is based upon the association of alopecia areata with other auto-immune diseases such as hypothyroidism, and the increased incidence of auto-antibodies in patients with alopecia areata compared with the normal population.[11,12] Histological evidence of an auto-immune basis is seen in the lymphocytic infiltrate around affected follicles.[13] The success of contact allergens in the treatment of alopecia areata is also indirect evidence of auto-immunity because their mode of action appears to be immuno-regulatory, rather than simply irritant.[14] It has been postulated that the response to contact allergens introduces non-specific T suppressor cells into the upper dermis which inhibits the lymphocytes associated with hair follicle damage.[15]

Our results suggest that DPC is probably no more effective than the other contact allergens, DNCB and SADBE in promoting hair growth; however, it does not have the disadvantages of mutagenicity in the Ames test or instability in solution.

The end-point in a trial of treatment for alopecia must be carefully defined. To report 'growth' or 'no growth' may produce impressive figures but gives little idea of the extent of the regrowth and, therefore, of the true benefit to the patient. We found our grading system for re-growth to be useful in this context; grades 1 and 2 represent limited re-growth which is of slight cosmetic value to the patient. Grade 3 may be cosmetically satisfactory alone or with the addition of a hair-piece, while grade 4, denoting a full head of hair, is the desired end-point.

In our study 50% of patients showed some re-growth varying from vellus hair only to a full head of hair. This compares only moderately favourably with the other contact allergens in which success rates of up to 80% have been reported. In Happle's work with diphencyprone[4] in which he achieved 65–70% success, the actual duration of alopecia was not given. Our group of patients had long-standing alopecia (mean 22 years) and we feel this may be the reason for the difference in our results, as we achieved only 29% cosmetic satisfaction (grade 4).

Success did not appear to be related either to the patient's age or the duration or extent of the alopecia. This suggests that neither the elderly nor those with extensive alopecia need be denied treatment, but it also means that there are no predictors for failure. At the moment, it must be assumed that those who fail to grow have a more active auto-immune process which is not reversed by DPC. Would 'failures' have done better had stronger concentrations been used to produce a more vigorous reaction? The answer is not known, but a more severe contact dermatitis would limit the appeal of the treatment. One patient with excellent grade 4 re-growth was prepared to spend 1 or 2 sleepless nights due to intense pruritus because she felt the extent of the pruritus correlated with her hair growth.

In the present study we attempted to maintain a moderate pruritus for 24 to 36 h after application on the basis that pruritus is a necessary symptomatic manifestation of the immune response. Botham et al.,[16] however, have reported that a low concentration of dinitrofluorobenzene in sensitized mice can produce a profound increase in cutaneous Langerhans cells, but with no associated erythema or tissue oedema. This raises the interesting possibility of sensitizing patients and then treating them with non-pruritic concentrations of DPC.

Diphencyprone is a treatment in which side-effects are to be expected. The resulting contact eczema ranges from a minimal itch to a severe reaction with blistering and weeping of the scalp and sometimes the neck, and periorbital oedema. The majority of patients who developed such reactions did so after treatment of the whole of one-half of the scalp with the 2% concentration. We subsequently modified our sensitization technique such that the 2% sensitizing dose was applied to only a small area about (4 × 4 cm) on one-half of the scalp and severe reactions were, therefore, limited and more easily controlled. It was not possible, retrospectively, to predict those patients likely to develop a severe reaction.

Diphencyprone is a treatment, not a cure, for alopecia areata and once a patient has responded, he or she will need long-term therapy. It may be possible to treat patients less frequently than once weekly, although one of our patients in whom this was attempted started to lose hair.

In this study 29% of patients with long-standing extensive alopecia regrew sufficient hair for it to be cosmetically acceptable. An unexpected finding was the psychological dependence upon their wigs displayed by several of the patients, to such an extent that they were initially unwilling to relinquish their wigs despite a full head of hair beneath them. Conversely, many patients gained great satisfaction from a lesser degree of regrowth as it allowed them the confidence to discard their wigs while at home. Some men might be surprised to find that they have progressed to male pattern balding which only becomes apparent after successful treatment.

We have found diphencyprone to be an effective stimulator of hair growth in patients with long-standing alopecia areata regardless of age. However, continuing evaluation of the long-term safety of this chemical is required, particularly if it is likely to become a permanent treatment for alopecia areata which could be lifelong.

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Disseminated superficial porokeratosis and immunosuppression

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SUMMARY

We present a patient who developed skin lesions typical of disseminated superficial porokeratosis (DSP) while on immunosuppressive therapy for pemphigus foliaceus. Phototesting with artificial light sources did not have any effect on the DSP lesions. The literature describing occurrence of DSP on immunosuppression is reviewed and possible pathogenetic mechanisms are discussed.

The term disseminated superficial porokeratosis (DSP) was introduced in 1937 by Andrews[1] to describe a clinical variant of Mibelli's porokeratosis.[2] Chernosky and Freeman[3] drew attention to a possible actinic aetiology of DSP and proposed the term disseminated superficial 'actinic' porokeratosis (DSAP). In contrast to Mibelli's porokeratosis, lesions of DSAP are smaller and more generalized, very often being confined to sun-exposed areas. The occurrence of DSP also was associated with artificial light exposure.[4,5] However, many subsequent reports lack adequate confirmation of actinic induction or exacerbation of skin lesions.[6] Immunosuppressive therapy as a contributing factor in the development of DSP was first observed by MacMillan[7] in 1974 and to the best of our knowledge about 16 further cases have been reported supporting the possibility that immunosuppression may be a cause of DSP.[8–14] We present a patient suffering from pemphigus foliaceus who developed typical DSP lesions on immunosuppressive therapy with prednisone and azathioprine. Phototesting was performed to determine whether this would exacerbate preexisting lesions or induce new lesions.

CASE REPORT

A 70-year-old male patient was first seen in our department in 1986. His history revealed malaria tertiana in 1943, dysentery in 1944 and meningitis in 1950. Because of a colon carcinoma in 1983 hemicolectomy was performed. Subsequent follow-up examinations revealed no signs of metastases or recurrence.

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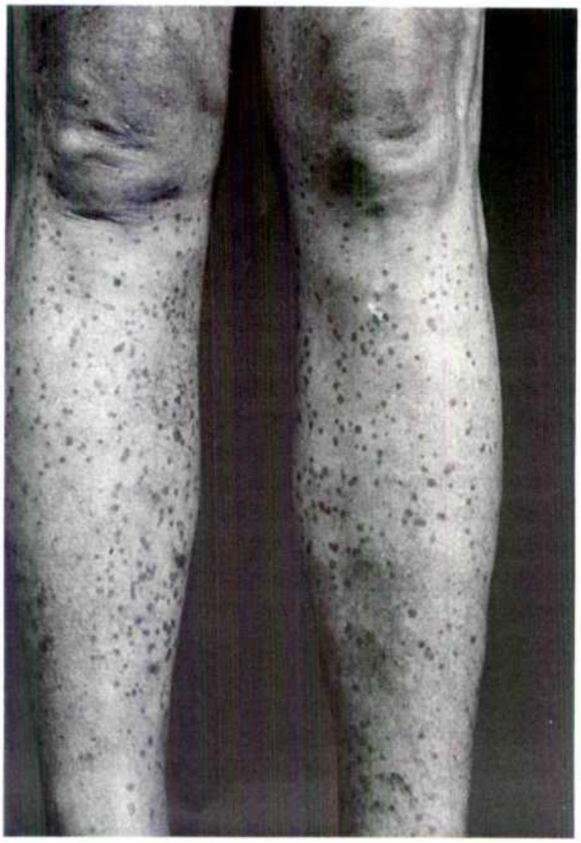

FIGURE 1. Multiple lesions of DSP on the lower legs.

At the first evaluation, pemphigus foliaceus, located on his trunk, was diagnosed, and treated with topical corticosteroids and azathioprine. A heart attack in October 1986 led to recurrence of his pemphigus foliaceus. Immunosuppressive therapy was resumed with 80 mg 6-methylprednisolone and two 50 mg doses of azathioprine daily and adjusted according to clinical improvement. In July 1987, while on azathioprine 50 mg/day and 4 mg 6-methylprednisolone/day he began to develop numerous lenticular brownish-red keratotic lesions on both legs, and some isolated lesions on his forearms and chest (Fig. 1). The lesions were surrounded by a slightly raised sharply defined ridge (Fig. 2). Besides itching, especially at night, no other symptoms were reported. The family history was negative. The patient reported having avoided sun-exposure during preceding years.

HISTOLOGY

Two punch biopsies were taken from the peripheral ridge of clinical typical lesions on the lower legs and were processed routinely. Paraffin sections stained with haematoxylin and eosin revealed focal areas of mild hyperkeratosis surrounded by a flat invagination of the epidermis. In the centre of this invagination a column of parakeratotic cells, the so-called cornoid lamella,

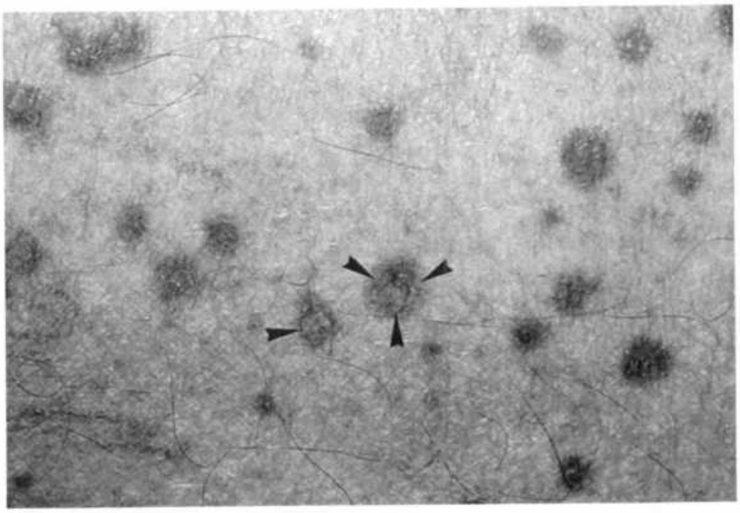

FIGURE 2. Close-up view of DSP lesions showing slightly raised keratotic borders (arrow heads).

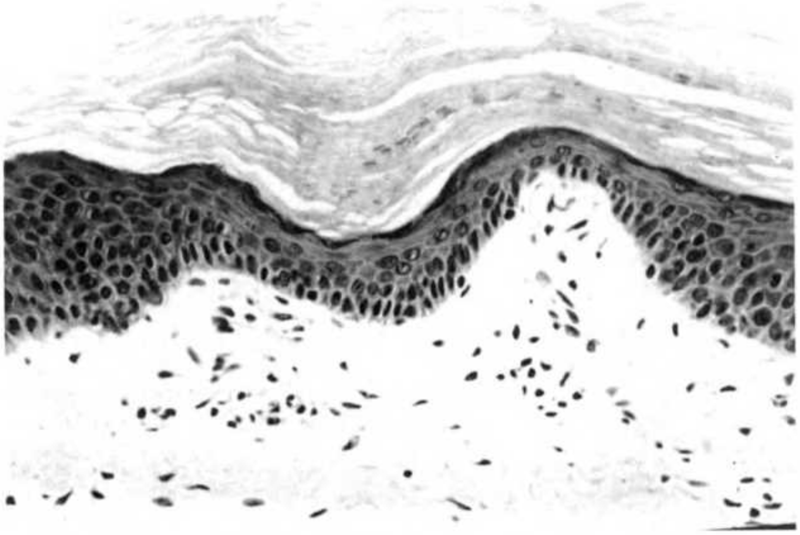

FIGURE 3. Histology showing typical cornoid lamella. (H&E, original × 180).

could be seen (Fig. 3). In the underlying dermis a mild lymphohistiocytic perivascular inflammatory infiltrate was present.

PHOTOTESTING

Despite a negative actinic history, phototesting procedures were performed to exclude any influence of light on the development of DSP.

UVA source

A high intensity UVA-device (SUPUVASUN 30 000, Mutzhas Co., Munich, FRG) was used. At a skin target distance of 70 cm the mean measured UVA intensity was approximately 80 mW/cm^2. The measurable irradiance was mainly in the 340–400 nm range, with almost no emission below 340 nm.

UVB source

A high-pressure mercury lamp (Osram Ultra-Vitalux, Osram GmbH, Munich, FRG) was used, emitting 48 μW/cm^2 as measured at 297 nm at a distance of 50 cm.

Two tests areas (each 7 × 14 cm), located on the extensor surface of the left lower leg and on the chest were irradiated on 5 consecutive days. One half of each test area was exposed to UVB, the other half to UVB plus UVA. One test area (7 × 7 cm) was exposed to UVA alone.

After 5 days of exposure with a total dose of 110 mJ/cm^2 UVB and 350 J/cm^2 UVA there were no new lesions or any change in pre-existing DSP lesions.

DISCUSSION

A correlation between immunosuppression and DSP is well documented in the literature (Table 1). Glucocorticosteroids alone[8,13] or in combination with azathioprine[7,12–14] or with cyclosporin,[9,14] and chemotherapy[9,12] have been reported to precede the first occurrence of DSP lesions in all these cases except one,[9] where preexisting DSP lesions were exacerbated during chemotherapy for acute myelogenous leukaemia.

Sites of predilection in most instances were sun-exposed areas, mostly the limbs, but lesions also occurred on body areas shielded from sunlight, for example the buttocks.[9,13]

TABLE 1. Previously reported cases of DSP on immunosuppression

Reference	No. of patients	Location of DSP lesions	Primary condition	Immunosuppressive therapy
MacMillan[7]	1	lower extremities	renal transplantation	prednisone 75 mg/day azathioprine 150 mg/day
Feuerman[8]	2	extremities, trunk	pemphigus vulgaris	prednisone 80–5 mg/day
Lederman[9]	2	legs, arms, back, buttocks	acute myelogenous leukaemia	daunorubicin cytosine arabinoside cyclosporine, corticosteroids
Bencini[10]	3	not reported	renal transplantation	methylprednisone 0·2–0·3 mg/kg azathioprine 1–1·3 mg/kg
Vire[11]	1	extremities	acute myelomonocytic leukaemia	daunorubicin cytosine arabinoside
Puissant[12]	3	not reported	mycosis fungoides primary biliary cirrhosis	immunosuppressive therapy plasma exchange
Bencini[13]	4	limbs, buttocks	renal transplantation	azathioprine 1–3 mg/kg/day methylprednisone 0·2–0·3 mg/kg/day
			chronic active hepatitis	prednisone 5–14 mg/day
Bouman[14]	1	extremities	renal transplantation	prednisone azathioprine cyclosporin 17·5 mg/kg/day

To exclude an actinic aetiology in the present patient, phototesting procedures were performed. This had led to severe exacerbation of skin lesions in two other patients, who gave a typical history of DSAP with worsening during the summer months (Neuman *et al.* unpublished data). In contrast, in the present patient, after phototesting, the skin lesions remained totally unchanged in size, colour and total number. The question of how light and immunosuppressive therapy can induce the development of DSP is controversial. Some authors proposed a latent skin change due to previous ultraviolet irradiation and suggested that subsequent immunosuppressive therapy might act as a second stimulus, resulting in DSP.[9] In contrast, Bencini[13] suggested that DSP development is strictly dependent on exposure to sunlight and that this irradiation probably amplifies the immunosuppression induced by drugs.[13] Our patient's history revealed only short periods of sun-exposure during the preceding years, and especially none during immunosuppressive therapy. Therefore, immunosuppression induced by prednisone and azathioprine seems to be responsible for the occurrence of DSP in this patient.

There is evidence that DSP is a genetic disorder with an autosomal dominant mode of inheritance.[15,16] Under certain circumstances a genetically determined aberrant cell population[17] might be activated and produce clinical symptoms. It is probable that the basic mechanism for the occurrence of DSP is immunosuppression in general, induced either by UV irradiation or by drugs. It appears also that other factors may be involved in the aetiology of DSP. Chernosky,[3] for example, observed an exacerbation of DSP lesions during the summer only in 14 of 31 patients, and Schwarz[6] demonstrated that more than one third of the patients studied presented no history of induction or exacerbation of DSP lesions by natural sunlight, although they had been diagnosed as having the 'actinic' form of DSP. The suggestion that infection, secondary to immunosuppression, might be of some importance in the aetiology of DSP has not been substantiated.[7,18]

A review of the literature and the features of our case clearly show that DSP is a disease with multiple aetiological factors, and that light exposure is not necessarily an initiating factor. We suggest that immunosuppression, regardless of the cause, is an important triggering mechanism.

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Necrotizing vasculitis and atherosclerosis

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SUMMARY

We describe five patients aged 50 years or above who had severe atherosclerosis and necrotizing vasculitis. The vasculitis was seen in the small vessels of four patients and in the medium-sized vessels of one. All five patients had multiple necrotic ulcerative skin lesions, and two underwent amputation. Our cases suggest that the relationship between the two disorders changes and exacerbates the clinical and pathological expression of each disease.

We have observed patients with combined necrotizing vasculitis and atherosclerosis and have found that both disorders seem to be altered by the concurrent disease. Combined clinical and histopathological evidence of inflammatory purpura and large- and small-vessel atherosclerosis may be found. The clinical course of the skin lesions is more progressive and destructive when both the vasculitis and atherosclerosis are present.

We report five patients who were found to have atherosclerosis with leukocytoclastic vasculitis.

METHODS

The records of five patients (three men and two women) with clinical and histopathological evidence of vasculitis and atherosclerotic disease were reviewed. The criterion for inclusion in the present study was the presence of leukocytoclastic vasculitis with fibrinoid necrosis of small or large vessels or both. All five patients had radiological or histopathological evidence or both of severe atherosclerosis, together with calcification of large arteries.

Skin biopsy specimens were obtained either by punch or excision. All tissue specimens were fixed in formalin and were stained with haematoxylin and eosin. The patients' clinical records were examined to assess the cutaneous and systemic features and the course of the illness.

RESULTS

Details of the patients are listed in Table 1. All five patients were more than 50 years old (mean age 66).

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TABLE 1. Clinical findings in five patients with necrotizing vasculitis and associated atherosclerosis

Case no.	Sex	Age (years)	Clinical findings	Histological findings	Associated disorders
1	M	69	Ulcerative skin lesions on lower legs	Small-vessel leukocytoclastic vasculitis	Severe atherosclerosis; myocardial infarction; diabetes mellitus
2	F	65	Small multiple ulcerative papules and plaques on lower legs	Small-vessel leukocytoclastic vasculitis	Severe atherosclerosis
3	M	77	Multiple small ulcerative lesions on lower legs	Small-vessel leukocytoclastic vasculitis	Atherosclerosis obliterans
4	M	52	Ulcers and nodules on lower legs	Small-vessel leukocytoclastic vasculitis	Atherosclerosis; myocardial infarction; diabetes mellitus
5	F	63	Multiple ulcerative papules and nodules on lower legs	Medium-sized vessel vasculitis; severe vascular calcification	Atherosclerosis; duodenal ulcer; chronic bronchitis

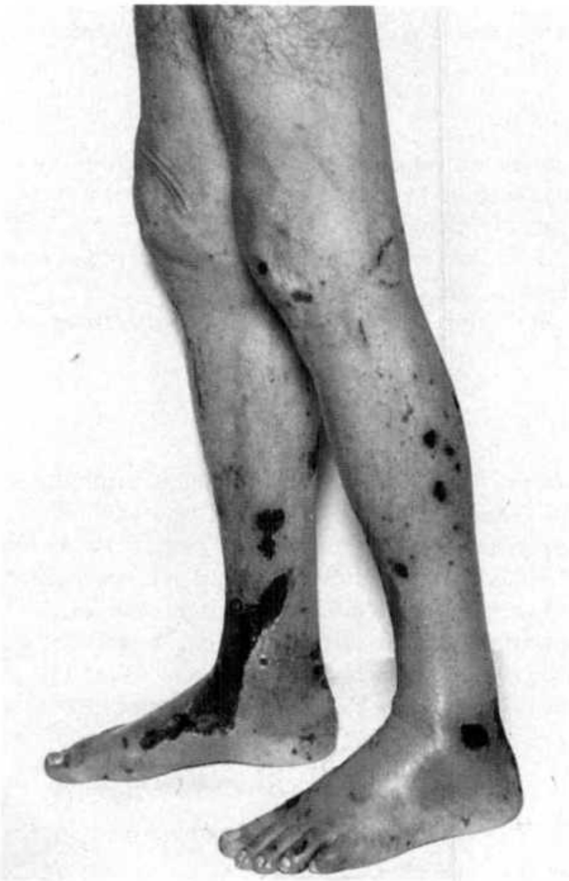

FIGURE 1. Multiple ulcerative skin lesions on the lower legs in patient with vasculitis associated with severe atherosclerosis.

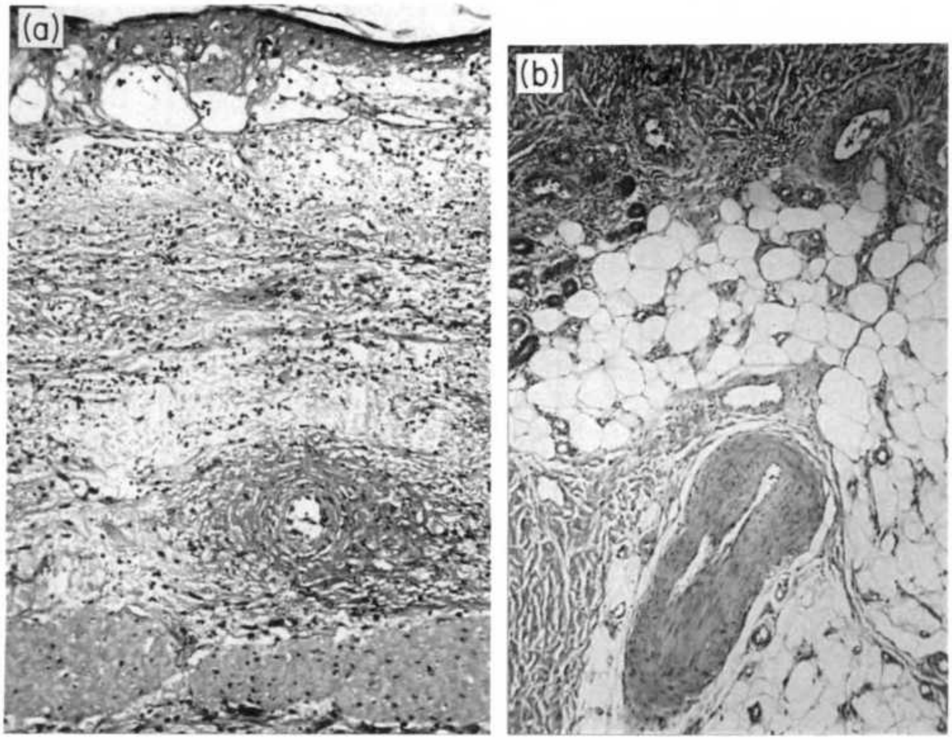

FIGURE 2. (a) Severe fibrinoid necrosis of dermal vessels with extensive epidermal necrosis. (Haematoxylin and eosin; original × 40.) (b) Endarteritis obliterans in close association with necrotizing vasculitis in a patient with severe atherosclerosis. (Haematoxylin and eosin; original × 64.)

Skin lesions had developed between 1 month and 1 year before the patients were seen, and were located on the lower legs. Generally, the lesions were multiple, erythematous, and purpuric. In each patient, the lesions developed into necrotic ulcers (Fig. 1).

In four cases, histopathological examination showed small-vessel vasculitis with polymorphonuclear leukocyte inflammation, nuclear dust, and fibrinoid necrosis. In two cases, there was severe necrosis of the epidermis (Fig. 2a). In one case, there was inflammatory involvement of medium-sized vessels in the deep dermis and the panniculus. One biopsy specimen showed endarteritis obliterans in close association with small-vessel necrotizing vasculitis (Fig. 2b). In these patients, severe ischaemic necrosis developed on the lower leg, and they underwent below-knee amputation.

All patients had evidence of atherosclerotic disease. Of the five patients, two had severe calcification of the abdominal aorta, two had aorto-iliac calcification, and one had renal artery calcification.

DISCUSSION

Necrotizing vasculitis or leukocytoclastic vasculitis is an immune complex vasculitis that is characterized by a destructive vascular and perivascular inflammatory purpura and by deposition of immunoglobulins and complement in and around the vessels in involved tissue.

The availability of extensive laboratory tests has resulted in a more accurate knowledge and classification of vasculitis. The major systemic aetiological associations of cutaneous vasculitis are infections, drugs, collagen diseases, and neoplasia.[1]

Circulating immune complexes are frequently found in vasculitis,[2] and some types of vasculitis, such as urticaria and mixed cryoglobulinaemia, are typically associated with low levels of circulating complement.[3]

The association of severe atherosclerosis with cutaneous vasculitis has been reported rarely and generally is related to cutaneous microembolization.[4,5] Evidence that atheromatous material may cause serological disturbances, such as complement activation *in vivo*[6], and polymorphonuclear leukocyte aggregation,[7,8] leading to endothelial damage, relates the presence of atherosclerotic disease to the development of vasculitis.

The differential diagnosis includes microembolization.[4,5] This diagnosis seems unlikely in our group of patients, as cholesterol crystals were not found and giant cells were not present in the inflammatory infiltrate. Nevertheless, this possibility cannot be totally ruled out as some studies have shown that intense eosinophilic infiltration of the vessel walls occurs soon after atheroembolization.[9]

The clinical cutaneous features of our patients were dramatic. At presentation, all five had multiple necrotic ulcerative lesions. This could be explained as a result of the additional factor of deficient vascular supply in regions with serious atherosclerotic occlusive disease of the main vessels. This cumulative phenomenon has been observed in experimental animals.[10] One of our patients had endarteritis obliterans in close association with necrotizing vasculitis. This form of vascular pathology occurs in response to atherosclerosis and intravascular thrombus formation[11] and is an additional factor in ischaemic injury.

We believe that it is important to recognize the association of neutrophilic vasculitis and atherosclerotic disease because the concurrence of both disorders alters the prognosis and progression of the disease. The response to treatment is similarly changed and the atherosclerosis limits the response to steroids and chemotherapy.

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Histiocytosis X in an elderly patient. Ultrastructure and immunocytochemistry after PUVA photochemotherapy

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SUMMARY

The clinical features of a 76-year-old man with histiocytosis X of the Letterer-Siwe type, with extensive skin involvement, are described. The patient's lesions responded dramatically to PUVA-photochemotherapy. Light microscopic, immunocytochemical and immunoelectron microscopic findings, before and after PUVA, are reported. Birbeck granules in phagolysosomes of dermal macrophages indicated uptake of destroyed HX cells. Residual HX cells in the skin and HX cells in a recurring lesion expressed the same membrane antigens as in the primary lesions.

Histiocytosis X (HX) is a proliferative histiocytic disease which is now also termed 'Langerhans histiocytosis'.[1,2] This disease presents a broad clinical spectrum from involvement of single organs to widespread cutaneous and visceral manifestations. Disseminated Langerhans cell histiocytosis of the Letterer-Siwe type is considered to be the acute form of infancy which almost always has a fatal outcome. This subtype is extremely rare in elderly patients where it may have a better prognosis.[3] At present it is not known whether the clinical variants result from different maturation of HX cells, as is the case in other lymphoproliferative diseases. It is also not known whether the phenotype of HX cells alters with therapy. Therefore, we have carried out ultrastructural and immunohistochemical investigations, before and after PUVA therapy, in a 76-year-old man with histiocytosis X of the Letterer-Siwe type. It was hoped that such a study would provide insights into the biology and prognosis of the disease.

CASE REPORT

A 76-year-old man had a papular scaly exanthema involving predominantly the trunk and

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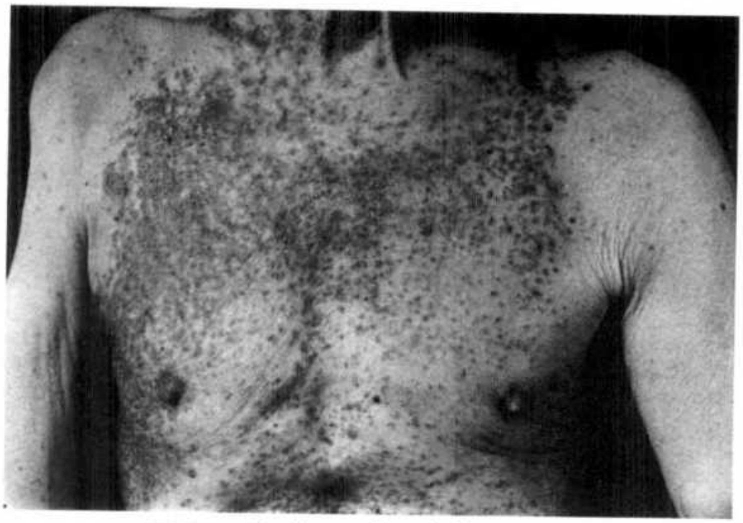

FIGURE 1. Papular exanthema before treatment.

extending to the extremities and the scalp. The skin lesions had been present for 18 months during which time they had steadily increased in number. The dermatosis had been treated with topical steroids and UVB without success. The patient complained of itching, but otherwise felt well. Physical examination showed an eruption of coalescing slightly purpuric reddish-brown papules involving most of the trunk (Fig. 1) the proximal flexures of the extremities and the temperoparietal area of the scalp. Many papules were covered by a crusted yellowish scale with a seborrhoeic-like appearance. There was neither lymphadenopathy nor hepatosplenomegaly. The lungs were clear to auscultation and percussion. Further physical examination was normal. On admission, protein electrophoresis showed an increase in gamma globulins (24·4%, normal: 10·5–19·8%). There was a polyclonal activation of immunoglobulin synthesis (IgA 975 mg/dl, IgM 299 mg/dl, IgG 2650 mg/dl and, IgE 207 mg/dl). Immunoglobulin electrophoresis of the serum was normal. White and red blood cell counts were within the normal range. A peripheral blood smear revealed slight lymphocytopenia (15%) and monocytosis (13%).

The erythrocyte sedimentation rate was increased at 31 mm in the 1st h. A chest X-ray was highly suggestive of lung involvement as it showed a fine reticular interstitial structure. A pulmonary function test gave almost normal results. A whole body scan (497 MBq^{99m}Tc-DPD) showed no bone involvement. A skull X-ray was normal. Ophthalmological investigation excluded exophthalmos. There were no signs of diabetes insipidus. Liver and spleen appeared normal on ultrasound investigation, and no abdominal lymph nodes were palpable.

Histology of two biopsy specimens showed clusters of large histiocytes with abundant eosinophilic cytoplasm and indented nuclei immediately beneath the epidermis. Focally the cells had invaded the epidermis and formed aggregates in the upper epidermal layers. Substantial formation of crusts was seen in these areas. Beneath the dermal HX cells there was a dense infiltrate consisting predominantly of lymphocytes.

TREATMENT AND CLINICAL COURSE

Systemic therapy was refused by the patient. Conventional photochemotherapy (PUVA) with

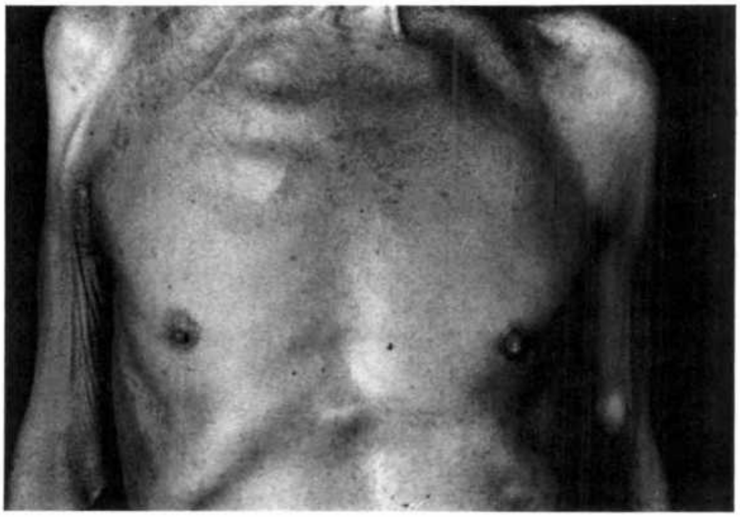

FIGURE 2. Clearance of lesions after 5 weeks of therapy with 8-methoxypsoralen UVA.

oral administration of 8-methoxypsoralen and UVA four times a week was started. Within 5 weeks of PUVA treatment (3·5 J/cm^2 four times weekly) the patient's skin lesions had totally cleared (Fig. 2) except for a few lesions on the scalp. Reduction of PUVA to one treatment per week was quickly followed by a relapse which was localized to the axillae and neck folds. Again the lesions promptly responded to increased doses of PUVA. Three months after admission, paraortic lymph nodes were found to be enlarged on ultrasound investigation and lymph nodes were palpable in the submandibular region. Another pulmonary function test and chest X-rays did not show progression of the disease. The patient still refused systemic therapy and further diagnostic procedures. The skin lesions were controlled by continuing PUVA therapy (4 J/cm^2, twice weekly).

METHODS

Punch biopsies (6 mm) were taken on admission, at clinical remission and on relapse. For electron microscopy the material was fixed in half-strength Karnovsky's solution, postfixed in osmium tetroxide and embedded in Epon 812. For immunoelectron microscopy, fresh tissue specimens were placed in Nakane's fixative (periodate-lysine-paraformaldehyde) for 2 h at 4°C.[4] After washing in 0·1 M phosphate-buffered saline with increasing concentrations of sucrose, the tissue was frozen in liquid nitrogen. Cryostat sections (40 μm) were incubated for 2 to 3 h with the monoclonal antibody T6 and then with a peroxidase-conjugated goat anti-mouse IgG (Dianova, Hamburg, F.R.G.) for 4 h at 4°C. The reaction product was visualized by 3,3′-diaminobenzidine tetrahydrochloride (Sigma, Deisenhofen, F.R.G.). The sections were postfixed in half-strength Karnovsky's solution and osmium tetroxide and embedded in Epon 812.

Part of the biopsy material was snap frozen and thin (6 μm) cryostat sections were incubated with the following monoclonal antibodies: T6, T4, T8 (Coulter Clone, Krefeld, F.R.G.), Leu4, HLA-DR (Becton Dickinson, Heidelberg, F.R.G.), KiM8 (Code BMA 0380, Behring AG, Marburg, F.R.G.), S-100, Ki 67 (Dianova), and anti-Il-2 receptor (Tac)-antibody (Dakopatts, Hamburg, F.R.G.) and staining was performed using a conventional indirect immunoperoxidase method.

RESULTS

Prior to PUVA almost all HX cells stained with T6 (Fig. 3) and T6-positive cells contained Birbeck granules (Fig. 4). Besides T6, the HX cells from our patient expressed HLA-DR, T4 and S-100, a set of membrane antigens which characteristically are present on normal Langerhans cells and HX cells.[5-7] In addition, the HX cells stained with T4, as has been reported in the few cases of Letterer-Siwe disease investigated previously.[8-10] HX cells did not stain with the pan-macrophage marker KiM8 nor could significant Ki67 or Il-2 receptor protein be detected by the method used here. After 4 weeks of treatment almost no HX cells were detectable. Immunohistochemical staining showed that T6-positive cells which also expressed HLA-DR had become rare in the dermis and were totally absent from the epidermis (Fig. 5). On electron microscopy, T6-positive cells proved to be residual HX cells (Fig. 6). In these sections Birbeck granules were seen within the phagolysosomes of dermal macrophages (Fig. 6, insert). Another biopsy was taken from a lesion which had recurred when PUVA temporarily had been

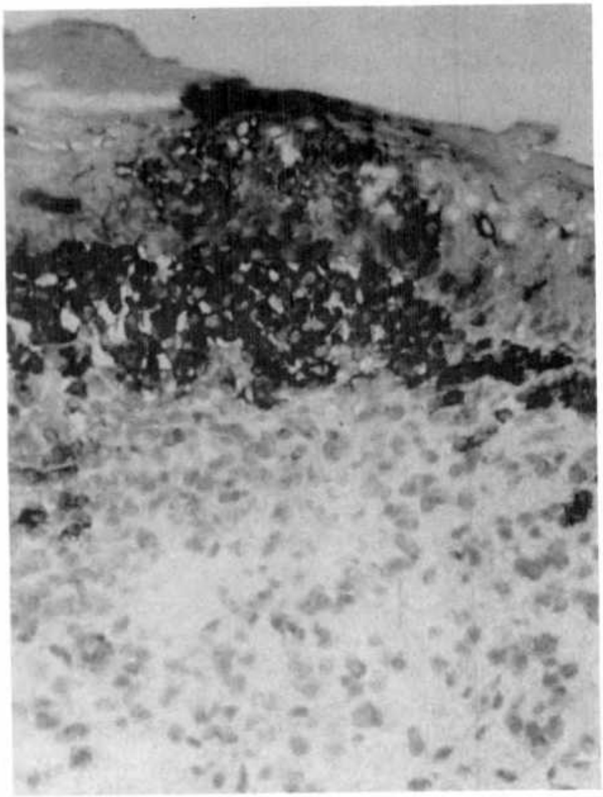

FIGURE 3. Dermal und intraepidermal histiocytosis X cells before treatment with 8-methoxypsoralen UVA. The cells stained intensely with the monoclonal antibody T6. (Counterstain: Meyer's haemalum; original × 205).

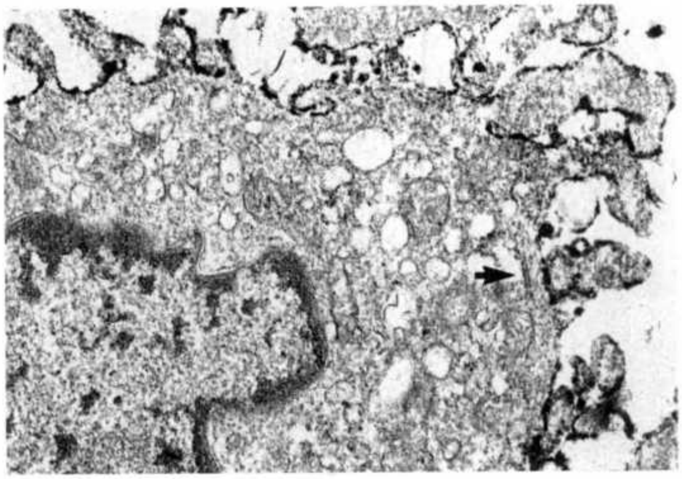

FIGURE 4. Immunoelectron microscopic demonstration of T6 antigen on the cell membrane of a histiocytosis X cell with Birbeck granules (arrow) before treatment with 8-methoxypsoralen UVA. (Original × 15 000).

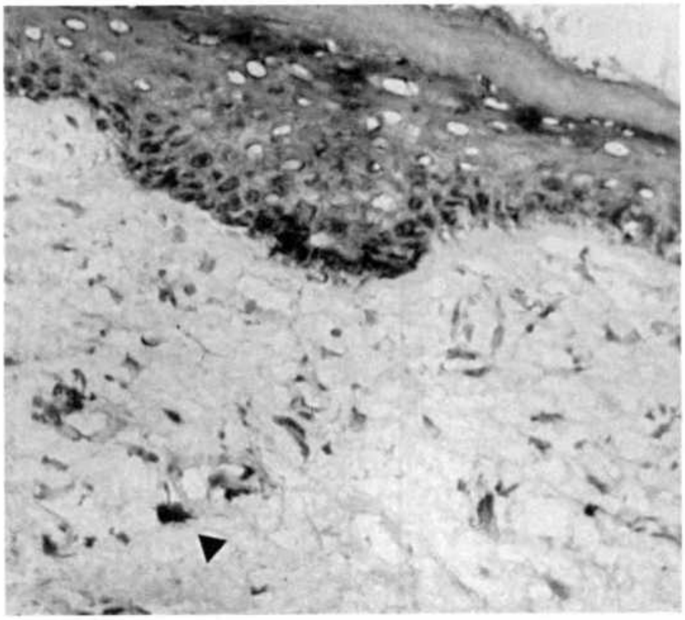

FIGURE 5. Absence of histiocytosis X (HX) cells from the skin after treatment with 8-methoxypsoralen UVA. A rare dermal HX cell is stained with the monoclonal antibody T6 (arrow head). (Counterstain: Meyer's haemalum; original × 205).

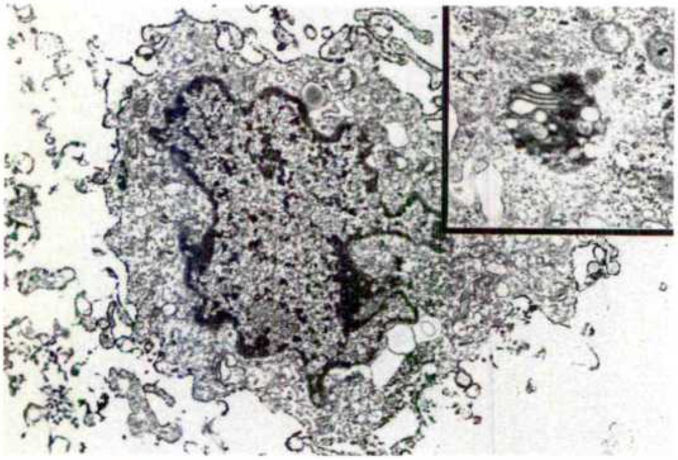

FIGURE 6. Demonstration of T6 antigen by immunoelectron microscopy on the membrane of a remaining tumour cell after treatment with 8-methoxypsoralen UVA. (Original × 6300). Inset: Phagolysosome of a dermal macrophage with Birbeck granules. (Original × 17 000).

reduced. Again nodular foci of subepidermal and intraepidermal HX cells stained with T6, T4 and HLA-DR, confirming stable expression of these membrane markers.

DISCUSSION

The clinical and histopathological features of the cutaneous lesions of our patient are similar to the Letterer-Siwe type of histiocytosis X occurring in early childhood. Hepatosplenomegaly, which is common in the childhood form, and rarely found in the elderly adults who have been reported to date,[11,12] did not develop in our patient. On clinical and radiological grounds lung and lymph node involvement was suspected, and this remained stable. During the observation period of 1 year, white and red blood cell counts did not point to bone marrow involvement. Although the erythrocyte sedimentation rate was consistently raised and the signs of polyclonal B cell activation with elevated IgA, IgG and IgM were increasing, the patient felt fairly well with no apparent progression of systemic disease during the observation period.

The cutaneous lesions in our patient responded promptly to PUVA therapy. It is known that Langerhans cells are highly sensitive to UV radiation. Using monoclonal antibodies and histochemical staining, other studies have shown that both the number of Langerhans cells, as judged by positive staining for ATP-ase, and the density of Ia-positive cells in the epidermis are decreased after UVB irradiation and after PUVA photochemotherapy.[13,14] Since we found Birbeck granules within phagolysosomes of macrophages it appears that these cells clear the UV-damaged HX cells from the skin.

Two further cases with cutaneous lesions of HX were recently described which were successfully treated with UVB or PUVA.[15,16] In the latter report the phenotype of the HX cells after PUVA therapy was also investigated. As with our case, a dramatic reduction of T6 positive dermal HX cells was observed and the residual cells had retained their membrane markers T6, OKIa1 and S-100 protein. Also, in the biopsy which we took from a lesion which had recurred after PUVA we were able to confirm the stability of marker expression.

Thus, the HX cells of adult onset disease and the disseminated childhood form seem to display the same set of membrane markers. HX cells appear to be destroyed by PUVA and eliminated by mononuclear phagocytes. The phenotype of remaining HX cells remains stable. To date there is no clear correlation between sites of involvement, rate of disease progression and available markers.

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Multiple basal cell carcinomas arising in port-wine haemangiomas

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SUMMARY

We report the case of a 49-year-old man, who had had two port-wine stains from birth, in which many basal cell carcinomas developed during his forties. The appearance of multiple basal cell carcinomas in port-wine stains has not been reported previously to our knowledge and may represent a new syndrome.

Port-wine stains (PWS) are present at birth and have no tendency to involution. They may be isolated lesions or be associated with other abnormalities including ocular (glaucoma), neural (Sturge-Weber syndrome) and abnormalities affecting bones and soft tissues (Klippel-Trenaunnay syndrome), thus constituting several entities,[1,2] some of them uncommon (Cobb, Wyburn-Mason and Beckwith-Wiedemann syndromes).[3,4] There is also an association of PWS with neoplasms such as renal carcinoma and phaeochromocytoma (Von Hippel-Lindau disease).[4,5] All these entities are well documented in major textbooks.[6,7]

There have been a few accounts of a single basal cell carcinoma developing within a PWS. We report here the case of a man with two PWS in which numerous basal cell carcinomas occurred. The possible aetiology of this association is discussed.

CASE REPORT

A 49-year-old Mexican man, with a dark skin, presented with two port-wine stains which had been present since birth; the larger one (12 × 6 cm) was on the left cheek and the smaller one (9 × 5 cm) on the chin (Figs. 1 and 2). Multiple (12) tumours developed in these lesions at the age of 40. These lesions were dome-shaped, dark brown, measured from 0·3 to 0·8 cm in diameter and were asymptomatic. They increased in size and number (up to 16) over the following years.

Clinical examination did not reveal any other abnormality. A full blood count, biochemical screen and urine and liver function tests were normal. X-rays of the entire skeleton showed no

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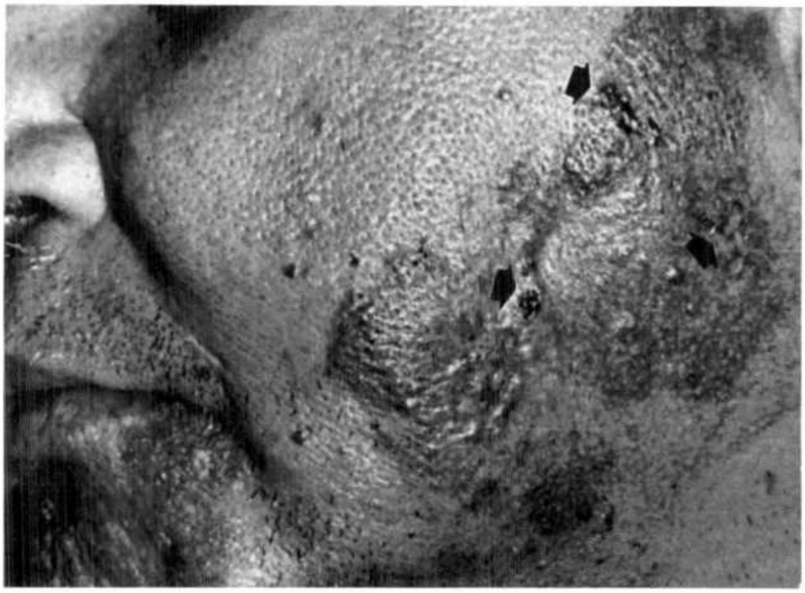

FIGURE 1. Patient's left cheek showing a port-wine stain on which multiple basal cell epitheliomas can be seen (arrows).

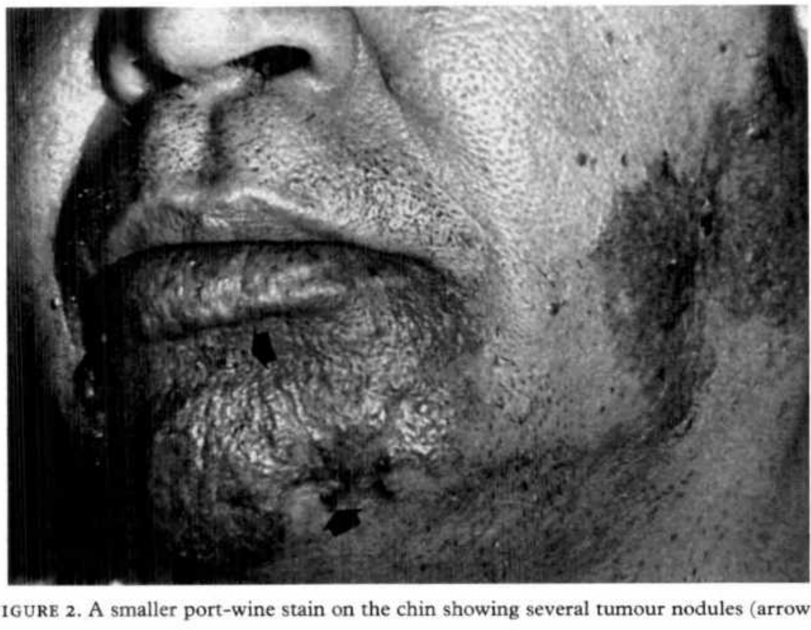

FIGURE 2. A smaller port-wine stain on the chin showing several tumour nodules (arrows).

abnormalities. The patient had not undergone any X-ray treatment nor did he report any other previous treament. He had no family history of a related disease.

Six excisional biopsies were performed; all of them showed the histological picture of basal cell carcinoma connected to the epidermis and with variable amounts of melanin. Dilated vessels were also seen in the histological sections (Fig. 3).

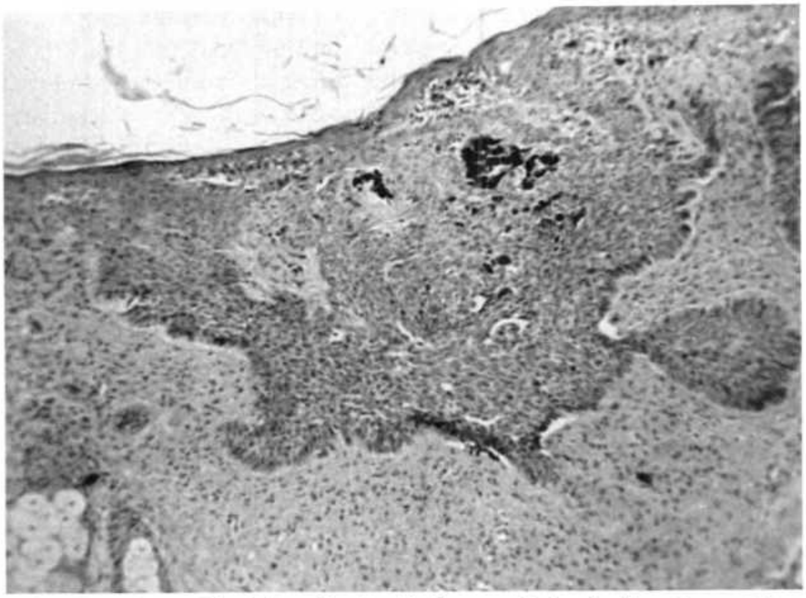

FIGURE 3. Histology of one of the lesions showing the features of a basal cell carcinoma. (Haematoxylin and eosin; original × 400).

The patient returned 1 year later with new tumour nodules with the same clinical and histological characteristics. He was referred to the Plastic Surgery Service.

DISCUSSION

Port-wine stains are congenital lesions, structurally characterized by ectasia of the superficial cutaneous vascular plexus, which is usually progressive and is accompanied by erythrocyte stasis. The overgrowth of vascular lesions in the field of a PWS is a well known phenomenon, and has been examined recently by Finley *et al.*,[8] who classified them into two groups: one characterized by proliferation and ectasia of thin-walled vessels of varying calibre, or of thin and thick-walled vessels with lumina of varying diameters (which they called arteriovenous malformation) and another group in which multiple polypoid lesions are formed by vessels with walls of diverse thickness and varying content of erythrocytes (cobblestone pattern). The content of erythrocytes determines the colour of the lesion. A cavernous component can also be found within PWS.

Basal cell carcinoma arises in skin damaged by solar and ionizing radiation and by other factors such as vaccination, burns, chronic leg ulcers or sebaceous naevi;[9] or may be associated with defects in DNA repair mechanisms, or with other cutaneous and osseous alterations as manifested in basal cell naevus syndrome.[10] Nevertheless, the development of basal cell carcinoma in PWS is exceptional and, to the best of our knowledge, only five cases have been documented to date. All of these are similar in that the lesion was solitary. The lesion reported by Martin-Scott,[11] which was the first to appear in the literature, occurred in a 38-year-old man on the abdominal wall, and in the case studied by Sarkany and Caron[12] the lesion was found on the right temple; neither patient had received previous treatment for the port-wine stain. There are three more cases in which the PWS had been treated either with thorium-X or with Grenz rays[11,13] with subsequent growth of a basal cell carcinoma.

The present case differs in that the basal cell carcinomas were numerous and increased in number, continuously arising in port-wine stains. This suggests that the ectatic vessels may produce an oncogenic factor, or make the overlying epidermis more susceptible to ultraviolet or Grenz radiation or the use of thorium-X; however, this cannot be substantiated at present. Nevertheless, it seems feasible to assume that the epidermal cells in the field of these PWS are somehow prone to mutation and subsequent neoplastic growth. We hope that presentation of this case will lead to greater awareness on the part of dermatologists of this association, leading to better understanding of the pathogenesis of this entity.

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Darier's disease and retinitis pigmentosa; is there a pathogenetic relationship?

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SUMMARY

Two brothers with Darier's disease and concomitant retinitis pigmentosa are reported. To our knowledge, these are the first cases with this combination of symptoms described in the literature. The dermatological conditions occuring with retinitis pigmentosa are reviewed. A disturbance in retinoids or retinoid binding proteins as a possible cause of both diseases is discussed.

Ophthalmological disorders are known to occur in Darier's disease. Of 21 patients, 16 showed corneal lesions.[1] Also, dark adaptation disorders have been described in patients with Darier's disease.[2] However, no patients suffering from both Darier's disease and retinitis pigmentosa have been reported, to our knowledge.

Retinitis pigmentosa is a degenerative disease of the retina, mostly with an autosomal recessive inheritance pattern, although X-linked recessive and dominant, and autosomal dominant forms exist. With onset in early childhood, patients suffer from bad vision at twilight, their visual fields are concentrically restricted and, depending on the type of retinitis pigmentosa, frequently total blindness develops.

We report here two brothers with Darier's disease and concomitant retinitis pigmentosa.

CASE REPORTS

Case 1

A 52-year-old man was first seen in 1979 at our clinic. The patient noted deterioration of night vision at 6 years of age. Later he had a traffic accident because he failed to see a vehicle. Retinitis pigmentosa had been diagnosed on the basis of typical retinal changes. At the age of 15, Darier's disease manifested itself for the first time. No other concomitant disease could be found. Family history revealed cataract in the father. Consanguinity was ruled out.

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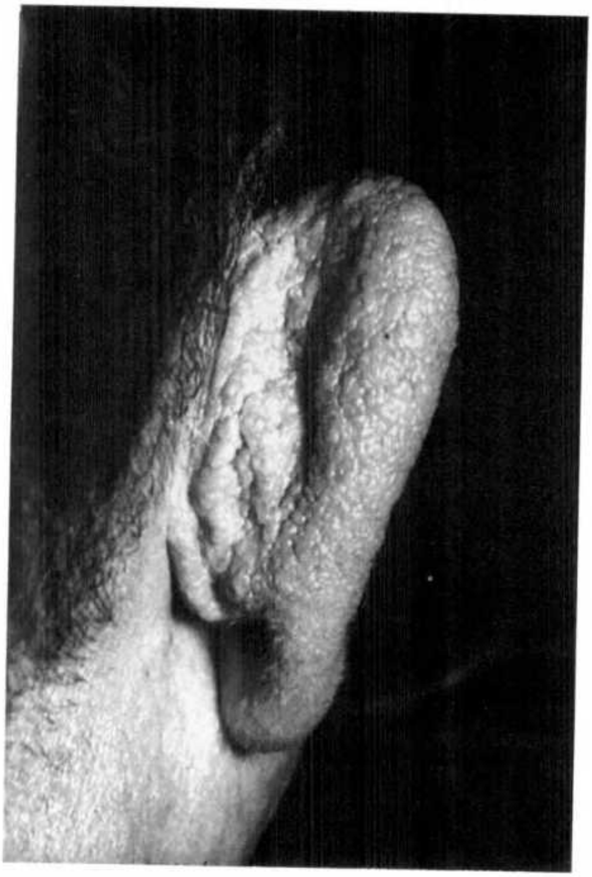

FIGURE 1. Case 1. Ear, showing papillomatous follicular keratosis.

On physical examination, his skin showed brownish papules, covered with a light brown scaly crust on the face, neck, shoulders, chest and back. The lesions coalesced into large plaques. Hypertrophic papillomatous warty masses were noted on both ears (Fig. 1). On the palms and soles minute pits were present. The nails were dystrophic with subungual thickening. The mucous membranes showed cobblestone-like papules.

Ophthalmological examination revealed a discretely excentric pupil in the right eye. The patient was aphakic after bilateral cataract extraction. There was bleeding into the vitreous with pale atrophic optic discs, narrow vessels and brownish-black pigmentation of the retina in both eyes. Visual acuity was reduced in both eyes to 0·05 and dark-adaptation was diminished. There was no response to an electroretinogram and the visual fields were less than 5 degrees. The remainder of the physical examination was normal.

A biopsy from the right ear showed hyperkeratosis, parakeratosis and papillomatosis of the epidermis with dyskeratosis resulting in the formation of corps ronds in the granular layer and grains in the horny layer. Suprabasal acantholysis and an irregular upward proliferation into the lacunae of papillae resulted in so called 'villi.' A lymphocytic infiltrate was present in the upper dermis (Fig. 2).

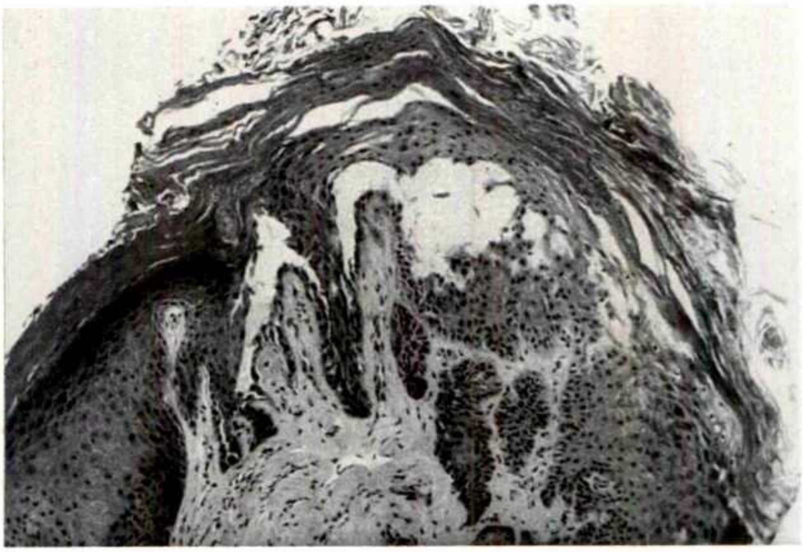

FIGURE 2. Case 1. Biopsy from the ear shows hyperkeratosis, parakeratosis and dyskeratosis of the epidermis with suprabasal acantholysis.

The patient was treated with topical steroids and etretinate (Tigason®) 25 mg/day. On this regimen the patient's skin lesions improved, and his condition remained well controlled for several years. Since 1961 he has taken vitamin A (90 000 IU/day) and vitamin E (210 mg/day) as well as beta-pyridyl-carbinol (150 mg/day) without improvement in the ophthalmological condition.

Case 2

A 48-year-old man, the brother of Case 1, presented to the dermatology service complaining of persistent scaling of the skin of his forehead and neck. The first signs of keratosis follicularis had developed during late childhood. A diagnosis of retinitis pigmentosa had been made at 33 years of age based on concentrically restricted visual fields and typical retinal changes. The patient was otherwise healthy.

Physical examination revealed scaly follicular papules on his neck, forehead and ears and in the auditory canal. Discrete hyperkeratotic lesions were present over the shoulders, chest and back. The nails showed white longitudinal bands and were quite brittle, and there were several pits on the palms.

On ophthalmological examination, the patient was noted to be aphakic following cataract extraction in both eyes. There were yellowish atrophic optic discs, narrow vessels and brownish-black pigmentation of the retina (Fig. 3). Corrected vision in the right eye was 0·25 and in the left 0·08. The visual fields were 10 degrees and dark adaptation was severely compromised. There was no response to an electroretinogram.

Skin biopsy revealed hyperkeratosis, parakeratosis and papillomatosis of the epidermis. Acantholytic cells were found in the lacunae. Corps ronds were present in the granular layer and grains were seen in the horny layer.

An improvement of the skin lesions was achieved with topical tretinoin 0·03%, carbamide

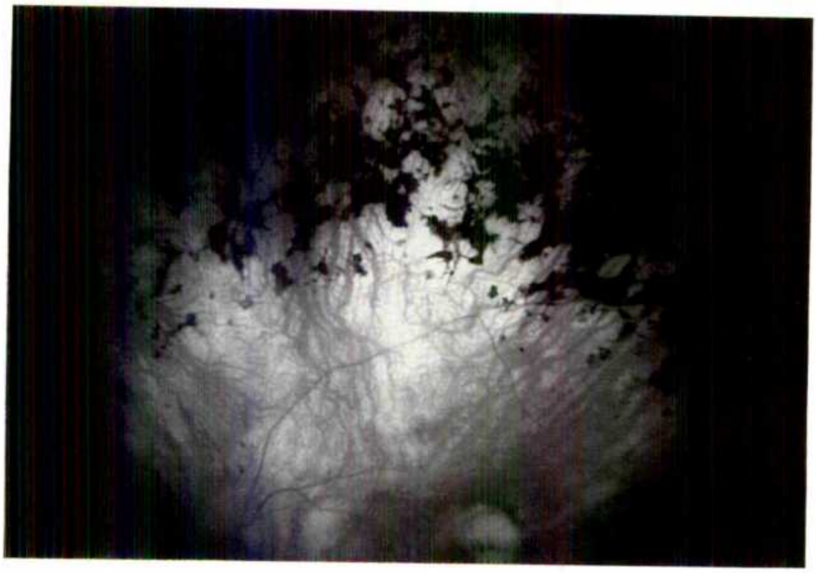

FIGURE 3. Case 2. The retina shows a pale optic disc, narrow vessels and brownish pigmentation.

12% and panthenol 1% in combination with systemic etretinate therapy (Tigason®, 25 mg daily). In addition therapy with vaccinium myrtillus (300 mg daily) and beta-carotene (15 mg daily) was given for ophthalmological reasons.

DISCUSSION

The present report of two brothers with Darier's disease (DD) and concomitant retinitis pigmentosa (RP) is the first description of this combination in the medical literature, to our knowledge. DD and RP are both rare diseases, so that the chances of a coincidence seem very small, particularly since two members of the same family suffered from an identical combination of symptoms. Although RP usually has an autosomal recessive inheritance pattern, X-linked recessive and dominant, and autosomal dominant inheritance have been documented. Keratosis follicularis follows an autosomal dominant inheritance pattern, with varying penetrance. The present combination of DD and RP raises the possibility that the genes concerned may be located next to each other.

RP is known to occur in association with various skin diseases (Table 1). Refsum's and Rud's syndrome can present with RP and ichthyosis accompanied by other symptoms.[3,4] In Cockayne's disease, RP has been found in conjunction with skin manifestations of photosensitivity, hyperpigmentation and loss of subcutaneous adipose tissue.[5] Porphyria cutanea tarda, naevus of Ota, vitiligo and alopecia areata have been reported in combination with RP.[6-9] Salamon[10] described a patient with RP and alopecia areata together with lingua plicata. Werner's syndrome, consisting of scleroderma-like changes and various systemic symptoms, may be combined with RP.[11] Basal cell naevus syndrome, Sturge-Weber syndrome and Letterer-Siwe disease have also been reported in combination with RP.[12-14]

The question arises as to whether there is a pathogenetic relationship between DD and RP. In RP as well as in DD, depletion of vitamin A has been discussed repeatedly. At present however,

TABLE 1. Skin diseases associated with retinitis pigmentosa

Skin disease	Reference
Frequently associated	
Refsum's syndrome	Thümler *et al.*[3]
Rud's syndrome	Larbrisseau and Carpenter[4]
Cockayne's syndrome	Liebermann *et al.*[5]
Rarely associated	
Porphyria cutanea tarda	Willerson *et al.*[6]
Naevus of Ota	Kumar and Singh[7]
Vitiligo	Cowan *et al.*[8]
Alopecia areata	Björk and Jahnberg[9]
Lingua plicata	Salamon and Stojakovic[10]
Werners syndrome	Valero and Gellei[11]
Basal cell naevus syndrome	Happle *et al.*[12]
Sturge-Weber syndrome	Berkow[13]
Abt-Letterer-Siwe disease	Francois and Bacskulin[14]

vitamin A deficiency as the sole aetiological factor is not considered a tenable explanation for the cause of either disease. It is not currently understood whether retinoids or retinoid-binding proteins play a definite role in the pathogenesis of RP or DD.

Lack of vitamin A leads to night blindness, an initial symptom in RP. Retinal degeneration develops and histologically resembles findings typical of RP.[15] Nonetheless high-dose vitamin A therapy does not improve RP.

Vitamin A deficiency leads to follicular hyperkeratosis of the skin, similar to the findings in DD. Impairment of dark adaptation has been described in isolated cases of DD.[2] In contrast to RP, Darier's disease usually shows improvement during vitamin A therapy.

Whereas retinol (the aldehyde of vitamin A) is important for visual function, retinol and vitamin A acid appear to play major roles in skin and keratinization.

In patients with RP, measurements of serum levels of vitamin A and retinol binding protein have been performed in several studies, with contradictory results. Campbell *et al.* found decreased serum vitamin A levels in comparison with a control group.[16-18] Although Rodger[19] and Shearer[20] confirmed these results, the majority of researchers found no significant differences in vitamin A, carotene or retinol binding protein levels between patients with RP and controls.[15] Bergsma[21] described a patient with RP and a significantly reduced level of interphoto receptor retinoid binding protein (IRPB). Rodrigues[22] recently reported a patient with RP of the autosomal dominant type who showed markedly reduced IRPB in the retina.

In DD a slight reduction in serum carotene has been found; however, levels of vitamin A and retinol binding protein were within the normal range.[23] Cellular retinoic acid binding protein was found to be significantly increased in skin lesions of Darier's disease.[24] Vahlquist's study of skin biopsies in DD showed higher retinol levels in uninvolved and lesional skin compared with controls. Dehydroretinol levels were higher in lesional skin than in uninvolved or control skin. The possibility of a disorder in vitamin A metabolism in DD was suggested.[23]

Further research is necessary to define the pathogenetic mechanisms and relationship of these two diseases. Any ocular or visual disturbances should be recognized and evaluated in all patients with Darier's disease.

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Effect of isotretinoin on carbamazepine pharmacokinetics

MADAM, Isotretinoin may have an inhibitory effect on microsomal mixed function oxidases,[1] and consequently reduce hepatic drug metabolism. Carbamazepine (CBZ) is extensively metabolized in the liver; one product is the active metabolite carbamazepine 10,11 epoxide (CBZ-E). Isotretinoin might, therefore, reduce metabolism of CBZ or CBZ-E or both and so could increase adverse effects from the drug or impair control of epilepsy.

A man of 23 required treatment with isotretinoin for severe acne. He had been treated for grand mal epilepsy with CBZ 600 mg once daily for 4 years with good control of seizures and without adverse effects. Before starting treatment with isotretinoin, concentrations of CBZ and CBZ-E were measured in plasma and saliva by HPLC.[2] An oral dose of 600 mg CBZ was given after an overnight fast, and samples were taken before dosing, and then at 1,2,3,4,6,8,12,18 and 24 h. These measurements were repeated after he had been taking 0·5 mg/kg/day isotretinoin plus 600 mg/day CBZ for 2 weeks; and after isotretinoin had been increased to 1 mg/kg/day plus 600 mg/day CBZ for a further 4 weeks. The concentrations of CBZ and CBZ-E before and during treatment with isotretinoin are shown in Figure 1. The elimination rate constant was calculated by least-squares regression analysis of the terminal elimination phase of the log serum concentration–time curve. The elimination half-life, distribution volume, clearance and area under the curve (AUC) (trapezoidal method) were calculated using a microcomputer program (STRIPE),[3] and are shown in Table 1 with values for CBZ-E AUC.

Plasma and salivary CBZ AUC decreased during treatment with isotretinoin; this could have been the result of decreased absorption or increased clearance, or both. CBZ clearance was increased during

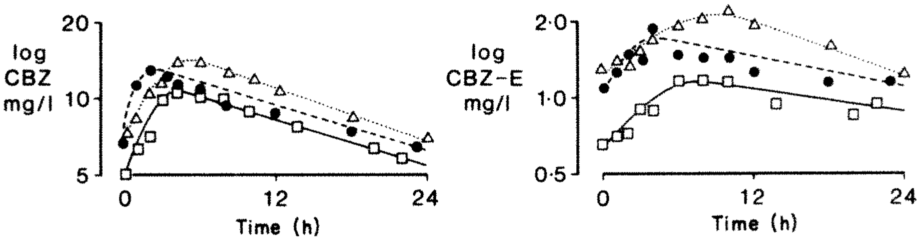

FIGURE 1. Concentrations of carbamazepine (CBZ) and carbamazepine 10,11 epoxide (CBZ-E) in plasma before treatment with isotretinoin (...△...) during treatment with 0·5 mg/kg/day isotretinoin (---●---) and during treatment with 1·0 mg/kg/day isotretinoin (—□—).

TABLE 1. Pharmacokinetic data for carbamazepine before and during treatment with isotretinoin

	Plasma					Saliva				
Isotretinoin	$t_{\frac{1}{2}}$	V_d	C	AUC	$AUC_{CBZ\text{-}E}$	$t_{\frac{1}{2}}$	V_d	C	AUC	$AUC_{CBZ\text{-}E}$
Before treatment	19·6	38	22·4	245	39	23·1	158	79	63	21
0·5 mg/kg/day	24·8	43	20·4	219	31	26·6	183	79	56	14
1·0 mg/kg/day	20·3	48	27·5	187	22	19·8	140	100	55	10

$t_{\frac{1}{2}}$ = half life (h), V_d = distribution volume (litres), C = clearance (ml/min.), AUC = area under concentration–time curve.

treatment with 1 mg/kg/day isotretinoin, but not with 0·5 mg/kg/day. The ratio of plasma CBZ AUC: CBZ-E AUC was 6·3 before isotretinoin, 7·0 with 0·5 mg/kg/day isotretinoin and 8·5 with 1 mg/kg/day, and was confirmed by ratios in saliva which were 3·0, 4·0 and 5·6, respectively. This could have been due to reduced metabolism of CBZ to CBZ-E, or to increased CBZ-E clearance or both; the latter could be determined by measurements of urinary clearance of CBZ-E.

These findings suggest that isotretinoin alters bioavailability or clearance or both of CBZ and CBZ-E. If both absorption of CBZ and its metabolism to CBZ-E are reduced, the effect on CBZ levels would be unpredictable, especially as the extent of CBZ metabolism to CBZ-E varies considerably between individuals.[4] Although our patient had no adverse effects, CBZ levels should be closely monitored during concurrent treatment with isotretinoin until further information is available about this possible drug interaction.

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Topical NDGA (nordihydroguaiaretic acid) in psoriasis

MADAM, We have recently reviewed the evidence implicating 5-lipoxygenase products in the pathogenesis of psoriasis.[1] Nordihydroguaiaretic acid (NDGA) is a plant product which is an antioxidant and a 5-lipoxygenase inhibitor.[2] The products of 5-lipoxygenase, leukotriene B4 (LTB_4) and 12-hydroxyeicosatetraenoic acid (12-HETE) are elevated in psoriatic skin. Since 5-lipoxygenase inhibitors may have therapeutic value in psoriasis, NDGA has been suggested as a possible antipsoriatic drug.[3] A recent study[4] confirmed that NDGA has an anti-lipoxygenase action *in vitro* and furthermore demonstrated a dose-dependent inhibition of keratinocyte growth in culture.

We, therefore, investigated the possibility that topical NDGA has a therapeutic effect in psoriasis *in vivo*. A modification of the therapeutic grid method described by Dumas and Scholtz[5] was used. NDGA was made up in a solution of industrial methylated spirit (80%) and water with ethylenediamine tetra-acetic acid disodium salt (0·01% w/w) as a chelating agent, to provide a stock solution containing 6% w/v (200 μmol/l) NDGA. This concentrate was stored at 4°C. Immediately prior to application, four dilutions were made up to provide concentrations of 0·5%, 1·0%, 2·0% and 3·0% w/v.

Four patients with stable plaque psoriasis were recruited, and gave fully informed written consent. Only emollients had been applied during the preceding week. In each patient a large uniform plaque was selected. The skin was cleaned with water and xylene, and a grid was marked out using a marker pen. The grids each had six squares each 1 cm by 1 cm. The squares were separated by applied double-sided sellotape 1·1 cm wide (Fig. 1). Ten microlitre volumes of the four NDGA dilutions and of the solvent as a control were applied to the first five squares using a Finnpipette®. The sixth square was covered with 0·05 ml of full strength betamethasone 17-valerate 0·025% ointment (Betnovate®) as a positive control. The whole grid was then occluded using sticky transparent plastic film.

The sellotape and plastic film were removed at 24 h intervals, the grid was photographed, cleaned and the test substances reapplied and reoccluded, for 14 consecutive days. Each day the squares were examined and the skin was designated normal or psoriatic. The presence of steroid induced vasoconstriction was noted.

The Betnovate® treated squares showed vasoconstriction on day 2 or 3 and had the appearance of normal skin after 9 to 11 days (mean 10 days). The remaining five squares showed no change throughout the treatment period other than a reduction in scaling due to occlusion, which was evident throughout the occluded plaque including those areas beneath the double-sided sellotape.

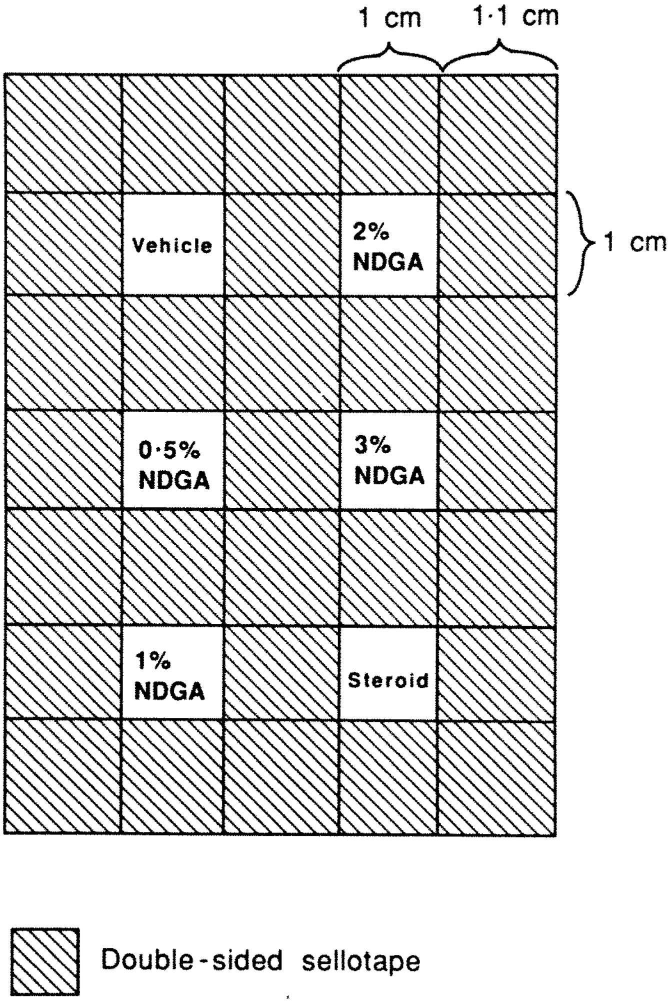

FIGURE 1. The therapeutic grid used in the study. The six test squares were delimited by double-sided sellotape, and the whole grid was occluded with plastic.

Thus, although there are good theoretical reasons to suppose that NDGA may be of therapeutic value in psoriasis, we have been unable to demonstrate this *in vivo*, despite using a range of dilutions and applying them for 14 days to involved skin. We now propose to measure the levels of lipoxygenase products in involved skin following NDGA application, since demonstration of a positive pharmacological inhibitory action in the absence of a clinical response would have important implications.

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Suitability of the upper back for corticosteroid vasoconstrictor assay

MADAM, The flexor aspect of the human forearm has been regularly used for ranking the potencies of topical corticosteroids in the vasoconstrictor assay.[1] Unfortunately, this convenient site has been shown to exhibit spatial variability in the blanching response to topically applied corticosteroids. A significant gradient effect has been demonstrated over the mid-portion of the forearm, with greater vasoconstriction recorded nearer the antecubital fossa.[2] The exact mechanism of this variation remains to be elucidated, but a spatial difference in reactivity of the local microvasculature to externally applied vasoactive stimuli, rather than differences in epidermal penetrability has been suggested.[3] This site-dependent variation on the forearm serves to introduce error in the vasoconstrictor assay and makes this convenient anatomical site less desirable. To date, similar variability has not been demonstrated at other anatomical sites. We, therefore, tested for this phenomenon on the skin of the upper back, as its absence would make this large and relatively hairless area more suitable than the forearm for future use in the corticosteroid vasoconstrictor assay.

Ten healthy volunteers (eight females, two males; aged 17–30 years) participated in the study after giving their informed consent. Patients with a history of skin disease or exposure to topical corticosteroids were excluded. Four Setonplast® templates each with three windows, measuring 3·5 cm by 1·5 cm and 1 cm apart, were applied horizontally across the skin of the upper back on either side of the midline at the level of the T_2 and T_5 vertebrae (Fig. 1). The middle window on each strip acted as the untreated occluded control. Desoxymethasone 0·05% w/w (Stiedex®) oily cream, 0·1 ml, was spread evenly with a wooden spatula onto the test sites, which were occluded for 3 h and then washed with soap and water. Cutaneous blanching was assessed before corticosteroid application and 8 h later, using reflectance spectrophotometry as described previously.[4]

Analysis of variance was used to analyse the differences between the four areas studied. Mean basal readings ($\pm$ SEM) were: right upper (R_1, R_2, R_3) 114·4 ± 6·2; right lower (R_4, R_5, R_6) 96·0 ± 6·7; left upper (L_1, L_2, L_3) 113·7 ± 7·2; left lower (L_4, L_5, L_6) 96·0 ± 5·5. The upper readings were significantly different from the lower ($P < 0·001$), but no significant right–left difference was found. In addition, a significant difference was found between medial and lateral sites: medial sites (R_1, R_4, L_1, L_4) mean ± SEM,

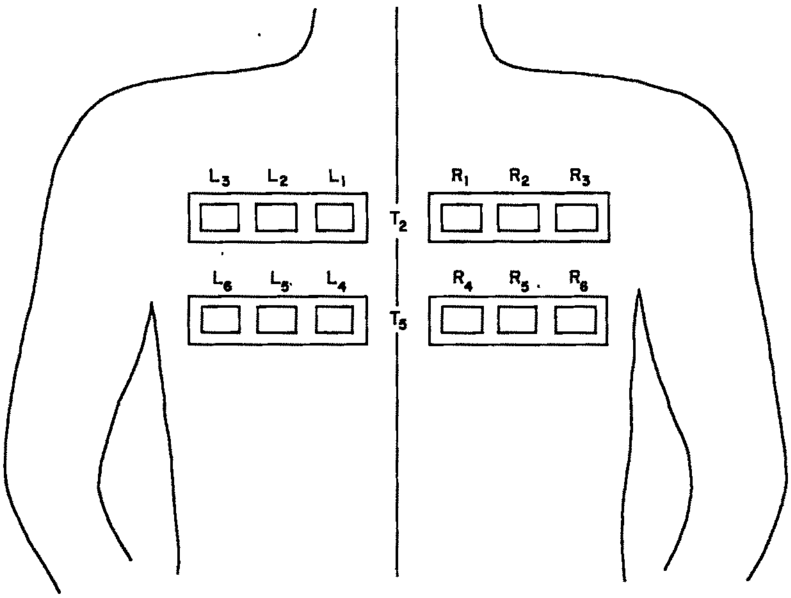

FIGURE 1. Relative positions of test sites on the upper back.

$110 \cdot 1 \pm 6 \cdot 2$; lateral sites ($R_3$, R_6, L_3, L_6) $98 \cdot 0 \pm 5 \cdot 3$ ($P < 0 \cdot 001$). All corticosteroid treated sites demonstrated obvious blanching at 8 h as compared with control sites, but the pre-steroid spatial differences were retained. These results are compatible with the view that the apparent spatial variability in the blanching response to topically applied corticosteroids is related to local differences in basal perfusion rather than to variations in reactivity of the microvasculature. As the upper back clearly offers no real advantage over the convenience of the forearm, the latter site should be retained for future corticosteroid vasoconstrictor studies. However, it is strongly recommended that a randomization plan[5] be used to avoid systematic errors arising from spatial variability in basal cutaneous perfusion.

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Danazol and discoid lupus

Madam, Lupus erythematosus has a diversity of clinical manifestations from the relatively benign to the severe and sometimes fatal systemic form of the disease. Although the aetiology is unknown, the tendency for it to affect females during their reproductive years suggests a hormonal influence on the clinical expression of the disease.

Danazol, a heterocyclic steroid with only weak androgenic properties has been shown to modify the disease activity in systemic lupus.[1] Its role, however, in discoid lupus has not been described previously, to our knowledge.

We would like to report a patient whose discoid lupus failed to respond to conventional therapy, but whose premenstrual exacerbations of disease activity responded well to hormonal manipulation with danazol.

A 47-year-old perimenopausal female first presented in 1980 with a 2-year history of a progressive scarring erythematous rash involving her face, ears and scalp. The erythema was noted to be more marked premenstrually. She was otherwise well and taking no medications. At no stage did she complain of mouth ulcers, musculoskeletal, serosal, neurological or renal symptoms.

A full blood count, erythrocyte sedimentation rate, liver function tests, serum creatinine, urinalysis, antinuclear antibodies, and antibodies to double-stranded DNA and extractable nuclear antigens were normal or negative. Skin biopsy showed features consistent with discoid lupus.

She was treated with sunscreens, topical steroids, hydroxychloroquine, and mepacrine serially or in combination, with little or no real benefit. The premenstrual flares of this patient's skin disease suggested to us that hormonal therapy might be of benefit in this patient. Danazol was commenced and the dose gradually increased to 400 mg daily. The skin lesions improved dramatically over the next few months, the only side-effects being amenorrhea and mild fluid retention.

To determine whether the patient's perimenopausal status was contributing to the remission she was asked to cease danazol. Three months later menstruation recommenced and the skin lesions again flared. Danazol was restarted and the skin lesions again dramatically responded. The improvement persisted and no new side-effects were noted.

Systemic lupus erythematosus occurs more commonly in females. The female to male ratio rises from 3:1 in childhood[1] to 9:1 after puberty.[2-4] This has led to speculation that the female hormones oestrogen and progesterone in some way modulate the phenotypic expression of the disease.

The immune system appears to be modulated by hormonal factors. Sex hormones have been shown to clearly modulate the immune response in the NZ B/W F1 hybrid mouse model of systemic lupus. Castration followed by replacement oestrogen therapy of female mice led to 94% mortality by 8 months. Substitution of oestrogens by androgens reduced the mortality to 14% in prepubertally castrated female littermates.[5] Prepubertal castration of the male NZ B/W F1 hybrid was followed by death in 100% by 11 months. In contrast the mortality rate among male littermates subjected to sham castration was only 17%. Androgens were shown to exert their effect not by reducing the concentration of anti-DNA antibodies, but by improving the clearance of immune complexes.[6]

Thus, in the murine model of systemic lupus, oestrogens appeared to augment, and androgens to retard the manifestations of the disease.

Morley *et al.*[7] described two patients whose systemic lupus was subject to premenstrual exacerbations. Introduction of danazol therapy was associated with a sustained remission in both. Premenstrual exacerbations of disease activity in discoid lupus have been reported,[8] but so far no hormonal manipulation of the disease has been attempted to our knowledge.

The results in our patient have shown that danazol may be useful in the treatment of premenstrual exacerbations of discoid lupus. Provided the patient's clinical status and liver function are carefully monitored, danazol may prove to be a useful and relatively safe therapy in some forms of discoid lupus resistant to other forms of therapy.

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Lack of effect of dimethyl sulphoxide (DMSO) on amyloid deposits in lichen amyloidosis

MADAM, The treatment of primary localized cutaneous amyloidosis has in general been disappointing. Dermabrasion has been used in some patients with some success,[1] but the benefit of etretinate seems to be uncertain.[2,3] Dimethyl sulphoxide (DMSO) is a penetrant carrier used to enhance percutaneous absorption of topically applied medicaments. Following a report that DMSO solubilized amyloid deposits in mice with casein induced amyloidosis[4] and two reports of renal amyloidosis improving with oral or parenteral DMSO,[5,6] other workers have investigated its potential in the treatment of localized cutaneous amyloidosis.[7,8] Bonnetblanc *et al.*[7] treated two patients with macular amyloidosis, with symptomatic improvement. In the single patient who underwent a post-treatment biopsy, deposits of amyloid were still present but some were fibrillar. Monfrecola *et al.*[8] treated one patient with lichen amyloidosis and recorded an improvement in pruritus as well as a flattening of the papules. Histological examination after treatment revealed partial disappearance of amyloid deposits in the papillary dermis. However, suggestions of a possible degredative effect of DMSO on amyloid deposits must be interpreted with caution since the pre-treatment and post-treatment biopsy sites were necessarily different. To settle this issue, we decided to conduct a simple *in vitro* experiment.

A 36-year-old Chinese man with typical lesions of lichen amyloidosis was selected. One papule was excised and snap frozen in liquid nitrogen. Four cryostat sections, 4 μm in thickness were cut and mounted on microscope slides. After air drying and rinsing in water, the first slide (the control) was stained with congo red. The second slide was immersed in pure DMSO for 3 days, the third slide for 5 days and the fourth for 1 week. After the respective periods, the slides were rinsed in water and then stained with congo red.

Examination of all the DMSO-treated specimens showed persistence of amyloid deposits. Comparison with the control slide also failed to reveal any fragmentation or partial disappearance of the amyloid deposits.

This study, therefore, shows that DMSO has no effect on established amyloid deposits in lichen amyloidosis. There appears little rationale for using topical DMSO for the treatment of macular and papular forms of primary localized cutaneous amyloidosis.

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Relapsing Polychondritis Support Group

MADAM, In 1981, at the age of 59, I was found to be suffering from a rare condition, relapsing polychondritis (RP), which brought my working life to an end. In 1986, by writing to dermatologists throughout England, I located 27 patients with the disease and through an announcement in the *British Medical Journal* contacted a patient in Eire. In January 1987 I formed the Relapsing Polychondritis Support Group, with eight members including myself.

It appears that an individual with RP first consults his general practitioner, and is then sent to an ENT

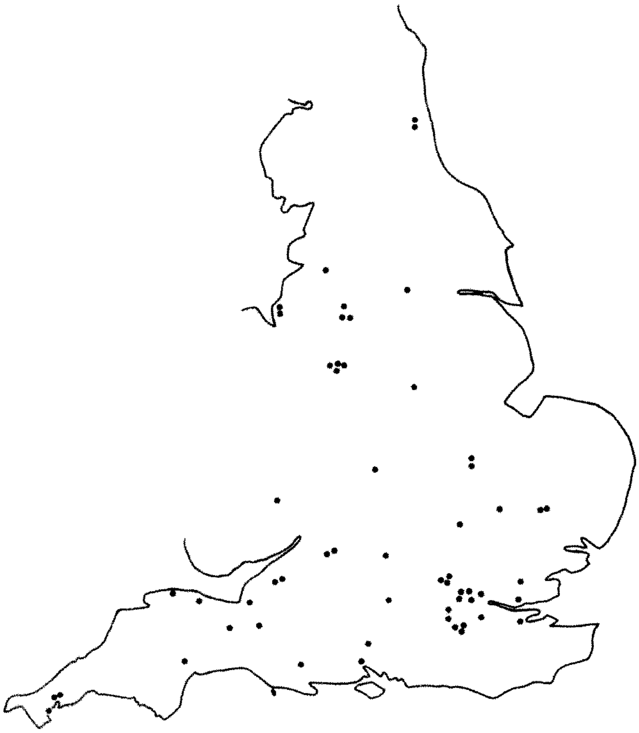

FIGURE 1. Locations of 57 relapsing polychondritis patients in England. March 1988.

specialist or an eye specialist, or both, or to a chest physician, usually ending up under the care of a dermatologist or a rheumatologist.

In order to locate the remaining RP patients in England, I contacted rheumatologists throughout the country and in 1987 located 30 further patients with RP. Seven joined our support group bringing our membership to 15.

The map in Figure 1 shows the location of the 57 RP patients, which might be of value in future epidemiological studies of the disease (further details available from the author).

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Erosive pustular dermatosis of the scalp—a definition

MADAM, Jacyk (*British Journal of Dermatology* 1988; **118:** 441) described six young African patients with scalp ulceration which he suggested 'may well represent a variant of non-infective erosive pustular dermatosis of the scalp occurring particularly in children and young adults'. This might or might not be true, but we think it is wrong at this stage to broaden the concept of erosive pustular dermatosis (EPD) without further evidence that this disease of African children is the same condition.

Lanigan and Cotterill[1] have similarly blurred this clinical entity by suggesting that the same condition occurs in the skin around leg ulcers due to venous hypertension, despite the fact that the histology in their cases showed dermatoliposclerosis rather than the histology of erosive pustular dermatosis of the scalp.[2]

The case for defining diseases as clearly as possible has been argued elsewhere,[3] and since we do not yet understand the cause or pathogenesis of erosive pustular dermatosis of the scalp, it must be defined as a syndrome, i.e. by the simultaneous presence of several disease indicants. The decision as to which indicants are necessary and sufficient to define a particular syndrome is pragmatic, but not immutable, and can be changed in the light of new knowledge.[3]

Until further information becomes available, we suggest that erosive pustular dermatosis of the scalp should be defined as follows: 'Chronic extensive pustulation confined to the scalp of an elderly person, and leading to erosion and scarring alopecia. No recognized cause of pustulation is present, and the histology is non-specific, showing atrophy and chronic inflammation, with an infiltrate consisting mainly of lymphocytes, although plasma cells and foreign-body giant cells may be present'.

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British Journal of Dermatology (1988) **119**, 412.

News and Notices

Dermatopathology Self-Assessment Workshop

A Dermatopathology Self-Assessment Workshop will be held at Guy's Hospital, London, U.K., 18–19 April 1989. The course is for both pathologists and dermatologists and will cover a broad range of subjects. Participants will have an opportunity to study 100 unknown slides during two microscope sessions, followed by a formal discussion. There will be a fee of £75·00 for all participants. The course is approved by the British Society of Dermatopathology. For further information contact: Marion Ellis, Secretary to Dr D.M.MacDonald, Dermatology Department, Guy's Hospital, St Thomas Street, London SE1 9RT, U.K.

An International Update in Dermatology and Dermatopathology

An International Update in Dermatology and Dermatopathology, organised by the Pathological Society of Great Britain and Ireland and the British Society for Dermatopathology, will be held in Brighton, U.K. from 2–5 September 1989. The meeting will include sessions on the following topics: skin appendage tumours (Chairman Dr N.P.Smith), non-epithelial tumours (Chairman Dr J.T.Headington), melanocytic tumours (Chairman Professor R.M.MacKie), pathogenesis of inflammatory dermatoses (Chairman Professor N.A.Wright), cutaneous lymphoid infiltrates (Chairman Dr A.B.Ackerman), immunologically mediated skin diseases (Chairman N.Kirkham) vasculitides (Chairman Professor E.Wilson Jones) epidermolysis bullosa (Chairman Dr P.H.McKee) and acquired bullous dermatoses (Chairman Professor M.W.Greaves). There will be two slide seminars. Further details available from: Dr P.H.McKee, Department of Histopathology, St Thomas's Hospital Medical School, Lambeth Palace Road, London SE1 7EH, U.K.

Advance Meeting Announcement
Ninth International Symposium on Contact Dermatitis

The ninth International Symposium on Contact Dermatitis will be held in Stockholm, Sweden from 17–19 May 1990. All those attending will be expected to give a short oral presentation or present a poster. Invited speakers will deliver review papers on recent advances in the field of contact dermatitis. Further information on the programme and instructions for the submission of abstracts will appear in September 1989. Symposium organizer: Professor Jan E.Wahlberg, Department of Occupational Dermatology, Karolinska Hospital, S-10401 Stockholm, Sweden.

69th Annual Meeting of the British Association of Dermatologists

The 69th Annual Meeting of the British Association of Dermatologists will be held at Newnham College, Cambridge, U.K. from 5–8 July 1989 under the Presidency of Dr R.H.Champion.

Abstracts are invited for consideration of the Selection Committee. These should be submitted on official abstract forms which are available from Dr M.M.Black, Honorary Secretary, British Association of Dermatologists, 6 St Andrew's Place, Regent's Park, London NW1 4LB. The closing date for receipt of completed abstract forms is 11 January 1989.

British Journal of Dermatology (1988) **119,** 413–418.

Centenary Celebration Section

The Editor's Prologue 1988

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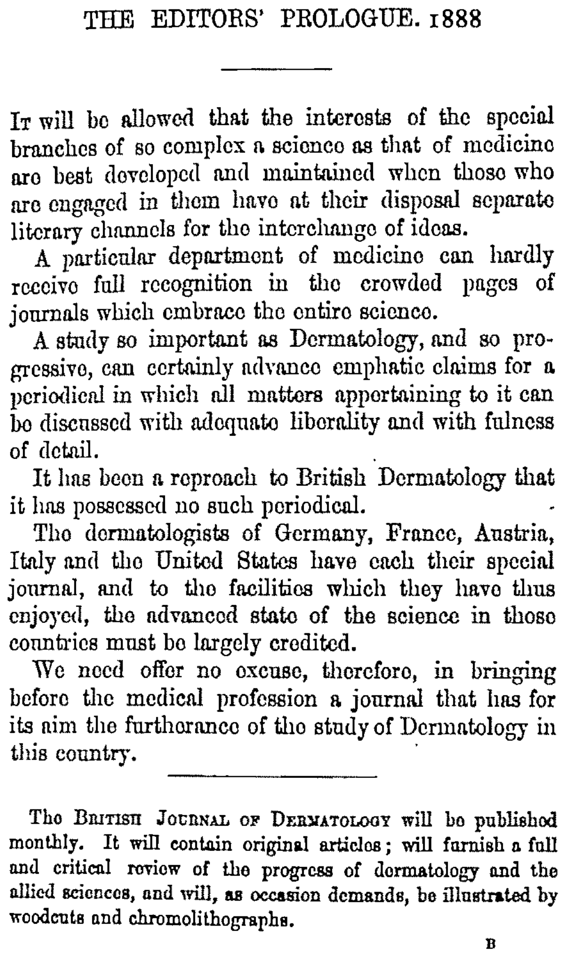
In this issue we celebrate 100 years of publication of the British Journal of Dermatology. Figure 1 shows the Editor's prologue written in 1888 to justify the birth of the Journal. The midwives in 1888 were Drs M. Morris and H.G. Brook. In their prologue they obviously feel the need to justify the introduction of a specialist Journal, and 100 years on it is very clear from a brief glance at the medical journal shelves of any large library that large numbers of potential Editors have

FIGURE 1.



THE EDITORS' PROLOGUE. 1888

IT will be allowed that the interests of the special branches of so complex a science as that of medicine are best developed and maintained when those who are engaged in them have at their disposal separate literary channels for the interchange of ideas.

A particular department of medicine can hardly receive full recognition in the crowded pages of journals which embrace the entire science.

A study so important as Dermatology, and so progressive, can certainly advance emphatic claims for a periodical in which all matters appertaining to it can be discussed with adequate liberality and with fulness of detail.

It has been a reproach to British Dermatology that it has possessed no such periodical.

The dermatologists of Germany, France, Austria, Italy and the United States have each their special journal, and to the facilities which they have thus enjoyed, the advanced state of the science in those countries must be largely credited.

We need offer no excuse, therefore, in bringing before the medical profession a journal that has for its aim the furtherance of the study of Dermatology in this country.

The BRITISH JOURNAL OF DERMATOLOGY will be published monthly. It will contain original articles; will furnish a full and critical review of the progress of dermatology and the allied sciences, and will, as occasion demands, be illustrated by woodcuts and chromolithographs.

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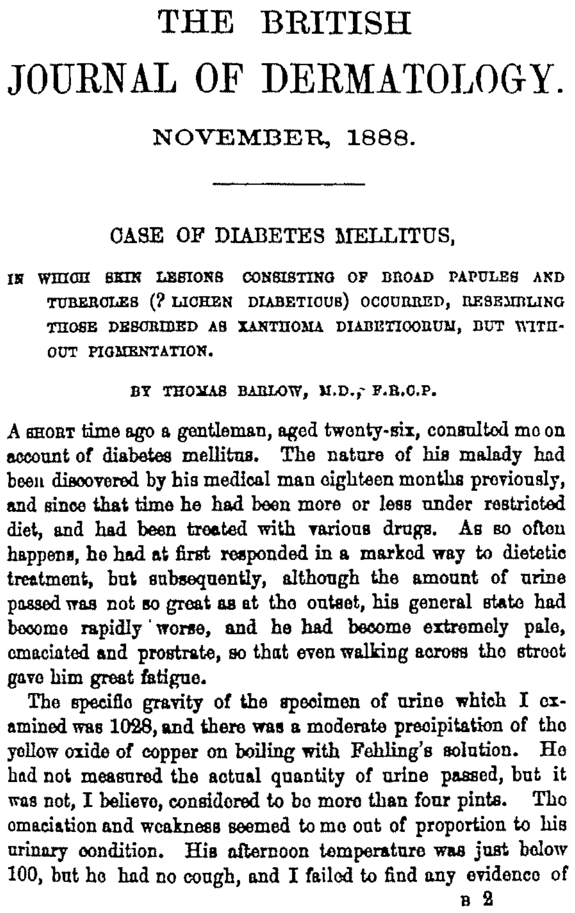
also felt this need over the past hundred years. Every six months the journal *Nature* publishes a half-yearly review of new journals which regularly includes some 20 to 30 additions to the Medical and Scientific literature. I am not aware of a similar list of defunct periodicals, so one can only assume that the upward rise in numbers of journals and thus numbers of scientific and medical communications published is at present continuing. In view of the current concern about funding for biomedical research, this is reassuring. Presumably, a time must come when this trend levels off or even declines, as there must be a final limit to the volume of literature which any individual can hope to digest. At a time when desk-top publishing and similar experiments in rapid communication are in vogue, it is of interest, and perhaps a matter for regret, that one of the recent rapid publishing experiments, that of Clinical Notes on Line, had a short life and has recently been withdrawn. There is no doubt that new approaches to rapid dissemination of information are required, as at the present time few monthly journals can publish a new and exciting communication in less than 6 months. This time interval, along with the current surge of interest and developments in molecular biology, is inappropriate in terms of the speed of emergence of new observations.

The confidence of the Editors in their prologue in 1888 is impressive. One hundred years on, I am sure all readers of the British Journal of Dermatology would concur with the importance and progressive nature of dermatology. The range and quality of papers submitted to, and subsequently published in, the journal at the present time would confirm that in 1988 the robust faith expressed in 1888 was not misplaced. It is of note that the British Journal was established after the birth of similar journals in four European countries and in the United States, at a time when internationally recognized dermatologists such as Radcliffe Crocker and McCall Anderson were publishing their excellent textbooks. The last sentence in the 1888 prologue suggests that the editors at that time envisaged the journal as being the mouthpiece of British dermatologists. It is good to note that over the last 100 years, communication around the world has become much more rapid, and today the *British Journal of Dermatology* is a publishing medium for the international dermatological community. During the calendar year 1987 approximately half the submitted manuscripts did in fact come from the U.K., but no less than 190 were submitted from the rest of Europe, with substantial numbers from both the United States and Japan and smaller numbers from the Arab countries, India, Australia, Canada, South America, South Africa, Nigeria, Korea, New Zealand, China, the U.S.S.R., Hong Kong, Sri Lanka and Singapore.

As would have been expected, papers published in the early years of the BJD were essentially clinical (Figs 2, 3 and 4), and illustrations at that time did not depend on the latest photographic gadgetry. One wonders how long Figure 5 took to complete, and whether courses in dermatological drawing were offered to trainees in this specialty. Advertisements were verbose and enthusiastic (Figs 6 and 7). The last sentence in Figure 7 suggests that in 1888 safety was recognized as an important aspect of therapy, but one does wonder about efficacy. Figure 8 is the 1888 equivalent of our present news and notes section, advertising appropriate courses. The cost of undergraduate dermatology training in 1888 even allowing for significant inflation was clearly significantly less than for overseas undergraduates and postgraduates at the present time.

Having surveyed the past 100 years, what can editors of the future expect? I would predict that the next 100 years will see much greater change in the format of dissemination of dermatological information than the last 100 have revealed. It would seem likely that with the information explosion, individuals may require to select for themselves rather more specific areas in which they plan to keep abreast of the literature, and to seek frequent updating via computerized literature searches and similar approaches in these chosen fields. It is clear that

FIGURE 2.



THE BRITISH JOURNAL OF DERMATOLOGY.

NOVEMBER, 1888.

CASE OF DIABETES MELLITUS,

IN WHICH SKIN LESIONS CONSISTING OF BROAD PAPULES AND TUBERCLES (? LICHEN DIABETICUS) OCCURRED, RESEMBLING THOSE DESCRIBED AS XANTHOMA DIABETICORUM, BUT WITHOUT PIGMENTATION.

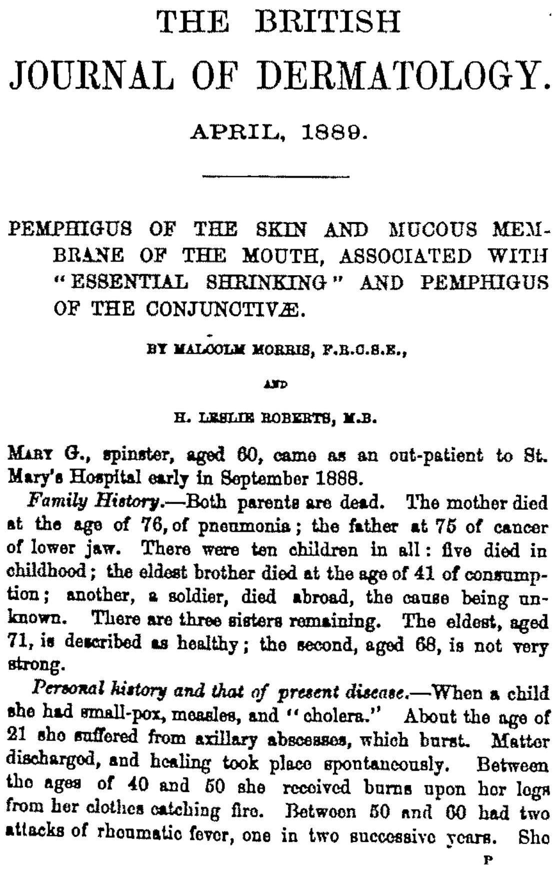
BY THOMAS BARLOW, M.D., F.R.C.P.

A SHORT time ago a gentleman, aged twenty-six, consulted me on account of diabetes mellitus. The nature of his malady had been discovered by his medical man eighteen months previously, and since that time he had been more or less under restricted diet, and had been treated with various drugs. As so often happens, he had at first responded in a marked way to dietetic treatment, but subsequently, although the amount of urine passed was not so great as at the outset, his general state had become rapidly worse, and he had become extremely pale, emaciated and prostrate, so that even walking across the street gave him great fatigue.

The specific gravity of the specimen of urine which I examined was 1028, and there was a moderate precipitation of the yellow oxide of copper on boiling with Fehling's solution. He had not measured the actual quantity of urine passed, but it was not, I believe, considered to be more than four pints. The emaciation and weakness seemed to me out of proportion to his urinary condition. His afternoon temperature was just below 100, but he had no cough, and I failed to find any evidence of

B 2

FIGURE 3.



THE BRITISH JOURNAL OF DERMATOLOGY.

APRIL, 1889.

PEMPHIGUS OF THE SKIN AND MUCOUS MEMBRANE OF THE MOUTH, ASSOCIATED WITH "ESSENTIAL SHRINKING" AND PEMPHIGUS OF THE CONJUNCTIVÆ.

BY MALCOLM MORRIS, F.R.C.S.E.,

AND

H. LESLIE ROBERTS, M.B.

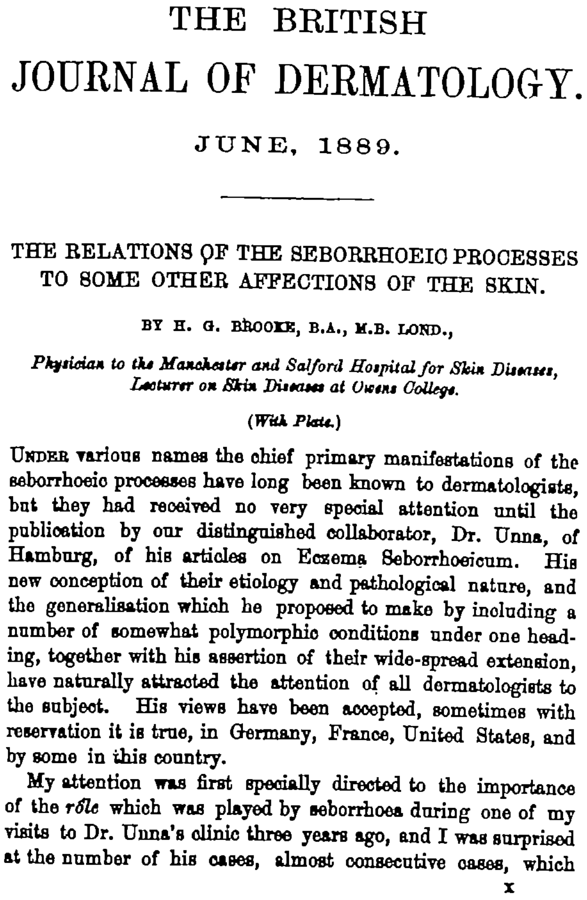
MARY G., spinster, aged 60, came as an out-patient to St. Mary's Hospital early in September 1888.

Family History.—Both parents are dead. The mother died at the age of 76, of pneumonia; the father at 75 of cancer of lower jaw. There were ten children in all: five died in childhood; the eldest brother died at the age of 41 of consumption; another, a soldier, died abroad, the cause being unknown. There are three sisters remaining. The eldest, aged 71, is described as healthy; the second, aged 68, is not very strong.

Personal history and that of present disease.—When a child she had small-pox, measles, and "cholera." About the age of 21 she suffered from axillary abscesses, which burst. Matter discharged, and healing took place spontaneously. Between the ages of 40 and 50 she received burns upon her legs from her clothes catching fire. Between 50 and 60 had two attacks of rheumatic fever, one in two successive years. She

P

FIGURE 4.



THE BRITISH
JOURNAL OF DERMATOLOGY.

JUNE, 1889.

THE RELATIONS OF THE SEBORRHOEIC PROCESSES TO SOME OTHER AFFECTIONS OF THE SKIN.

BY H. G. BROOKE, B.A., M.B. LOND.,

Physician to the Manchester and Salford Hospital for Skin Diseases, Lecturer on Skin Diseases at Owens College.

(*With Plate.*)

Under various names the chief primary manifestations of the seborrhoeic processes have long been known to dermatologists, but they had received no very special attention until the publication by our distinguished collaborator, Dr. Unna, of Hamburg, of his articles on Eczema Seborrhoeicum. His new conception of their etiology and pathological nature, and the generalisation which he proposed to make by including a number of somewhat polymorphic conditions under one heading, together with his assertion of their wide-spread extension, have naturally attracted the attention of all dermatologists to the subject. His views have been accepted, sometimes with reservation it is true, in Germany, France, United States, and by some in this country.

My attention was first specially directed to the importance of the *rôle* which was played by seborrhoea during one of my visits to Dr. Unna's clinic three years ago, and I was surprised at the number of his cases, almost consecutive cases, which

x

FIGURE 5.

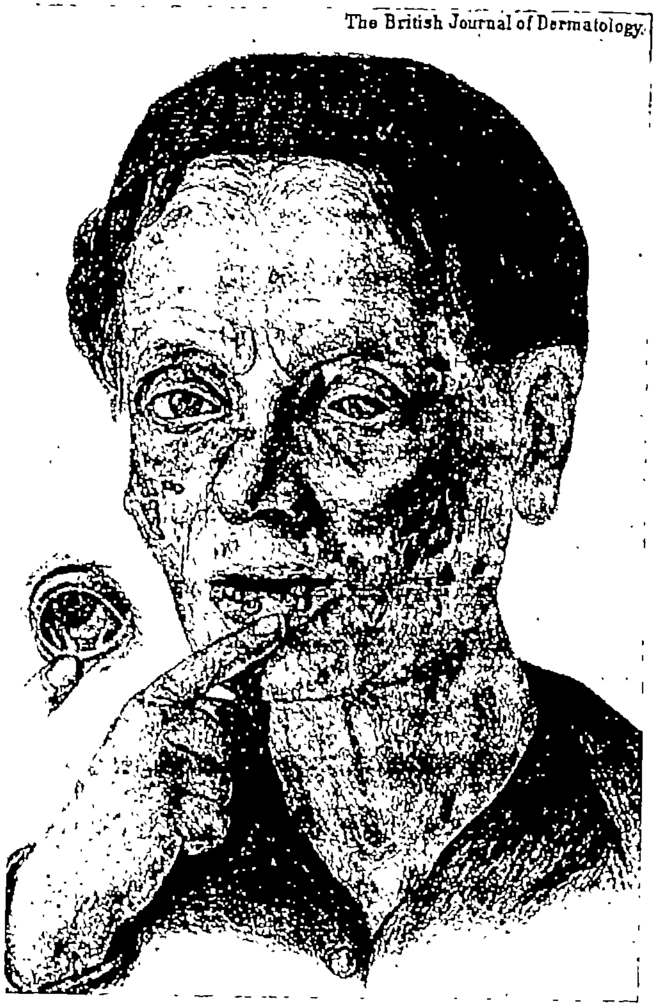


FIGURE 6.

SULPHAQUA.

THE HYGIENIC NASCENT SULPHUR BATH.

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BATH CHARGES, in boxes containing 1 dozen, 4s.
FACE-WASH ,, ,, ,, 2 ,, 3s.

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FIGURE 7.

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This powder is a simple, purified, natural product, which on analysis is found to be somewhat allied to superior varieties of Fuller's Earth. It contains a considerable percentage of Steatite, Silica, Alumina, and traces of Calcium Salts and Ferrous Oxide. The value of Emol-Keleet in the treatment of skin diseases has already excited great interest among the medical profession. In all cases EMOL-KELEET is perfectly harmless.

Supplied to the Medical Profession by all Chemists in boxes at 9d. each.

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FIGURE 8.

CLINIQUE ON DISEASES OF THE SKIN.

PROFESSOR McCALL ANDERSON will begin his CLINIQUE on Diseases of the Skin at the Hospital for Skin Diseases, Glasgow, on Monday, 28th April.

The Class will meet on Mondays and Wednesdays at 2.30 P.M., during the Summer Session, and there will be occasional written Examinations. Fee, £1 11s. 6d.

The Directors offer the sum of £5 to be expended on a prize or prizes to the most deserving Students, and other prizes will be awarded if warranted by the results of the Examination.

ARCHIBALD CRAIG, LL.B., HON. SEC.

the specialist journals covering the whole range of an area such as dermatology must be aware of these changing needs, and aim to adapt appropriately. Even in the next 20 years it is likely that there will be significant changes in the way in which we handle information to be disseminated and I, for one, look forward with great interest to seeing how my successor Stanley Bleehen and others will handle these changes. I wish them all well.

The following nine articles are invited reviews, all written by supporters of the BJD, each reviewing progress in a specific area over the past 100 years.

British Journal of Dermatology (1988) **119**, 419–426.

Centenary Review

Inflammation and mediators

M.W.GREAVES

Institute of Dermatology, St. Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, U.K.

INTRODUCTION

Although tissue destruction may be the end result, inflamed tissues retain their vitality enabling development of the classical features of inflammation: increased blood flow and vasodilation; accumulation of protein-rich exudate and cells; pain and itch. Chronic inflammation and healing cannot be separated from the pathophysiology of the acute inflammatory response, but they are essentially proliferative in nature, implying a different category of molecular mediators, and will not be considered further here.

That inflammation must be looked upon as a dynamic process and not as a fixed 'state' is a concept we owe to Metchnikoff (1892)[1] who vigorously asserted the then revolutionary concept of dynamic interrelationships of different types of participating cells, phagocytosis being a central phenomenon. Although inherently essential for the body's survival and protection, inflammation may be aberrant and considerably more harmful to the host than the insult which caused the reaction. Examples in skin include destructive forms of acne vulgaris and allergic responses.

To Cohnheim[2] we owe the first accurate description of the detailed microscopic morphological events occurring in inflammation. According to Cohnheim, the most important events in inflammation were vascular, involving changes in the vessel wall leading to margination and emigration of leukocytes and transudation of fluid. Cohnheim also related pain to the sensory nerves and heat to locally increased blood flow. Although Metchnikoff had hinted at the existence of chemoattractant mediators which draw white blood cells from the intravascular to the extravascular compartments, neither he nor Cohnheim appreciated the key role of leukotactic molecular mediators in inflammation. Modern studies of inflammation have mainly addressed themselves to this issue. Concepts of inflammation nowadays otherwise differ little from those held by the two nineteenth century investigators.

Research in inflammation during the 20th century mainly moved to a molecular level. The discovery by Paul Ehrlich[3] of the tissue mast cell and subsequent realization that it was a source of important molecular mediators, gave pharmacological research in inflammation increased impetus. Although mast cells had been known to contain heparin in their granules, realization that these cells, resident in the tissues, were an important source of pro-inflammatory mediators had to await the combined pharmacological and histometric studies of Riley[4] in the 1950s. Riley, who was a radiotherapist, established concordance between the population of heparin-containing mast cells and histamine content in a variety of tissues, including the skin, and specifically in urticaria pigmentosa. However, the importance of histamine as a mediator had been proposed well beforehand by Lewis[5] in the 1920s. The belief that chemical mediators play a major role in the inflammatory response probably owes its parentage to the early days of the science of immunology and the concept that humoral agents could, in the absence of cells, effectively combat microbial infection.[6] Lewis carried out a series of experiments in the skin, producing evidence that the central dull red flush of the 'triple response' due to firm stroking of the skin depended exclusively on chemical mediation, and he cautiously termed the mediator 'H-substance'. The similarity between this response and the response of skin to intracutaneous injection of histamine left Lewis in little doubt that histamine was indeed the mediator.

HISTAMINE

Histamine is characteristically a mediator of acute short-lived inflammatory responses. It is synthesized and stored in mast cells, its release being evoked by a variety of immunological and physical stimuli. In this sense mast cells can be looked upon as 'sentry cells', representing the first defence mechanism of the body to encounter with a noxious stimulus. That histamine is incapable by itself of evoking a long continued inflammatory response is suggested by the rapid development of tachyphylaxis in respect of the vascular responses in the skin elicited by histamine.[7] Direct evidence of the involvement of histamine in weal and flare reactions of the urticarial type has derived from skin perfusion studies carried out in patients with symptomatic dermographism, and analysis of venous blood draining cold-challenged skin in patients with cold urticaria.[8,9] In these studies there was a good concordance between the onset, development and regression of redness, wealing and itching and the concentrations of histamine found in the perfusate or venous blood. That histamine is not the sole mediator of the observed vascular changes is suggested not only by the incomplete response to antihistamine therapy, but also the observation that cold-evoking wealing is unaltered despite complete or almost complete drug-induced suppression of histamine release.[10]

Recent studies suggest the co-release with histamine of prostaglandin D_2, neutrophil and eosinophil chemoattractant peptides and proteases.[11–14] Nevertheless, the itching in urticaria is likely to be mainly, if not entirely, attributable to histamine.

Histamine release from human skin mast cells is a secretory process regulated by an adenylate cyclase–cyclic AMP-dependent mechanism.[15] The recent development of techniques for isolation of mast cells in suspension from human skin should lead to rapid progress in elucidation of the factors regulating release of mast cell mediators[16] and possibly lead to novel modes of treatment. Of particular interest is the recent discovery of a third sub-class of histamine receptors, designated H_3. The observation that H_3 receptors are represented in skin, and are associated with regulation of histamine biosynthesis and release, and the development of potent and selective ligands for histamine H_3 receptors[17] should lead to new insights into the role and modulation of histamine release in skin.

SHORT CHAIN PEPTIDE MEDIATORS

Two classes are of interest: the ubiquitious kinin system and the neuropeptides.

Kinins

Bradykinin, a nonapeptide, is the most widely investigated representative of this group and owes its name to its slow contractile action on the isolated guinea pig ileum preparation.[18] It is highly vasoactive in human skin, causing wealing, erythema and pain in nanogram doses.[7] Bradykinin is one of a group of peptides formed by the action of the esterase kallikrein on $\alpha 2$ globulin substrate. Because the kallikrein-kinin system is present in most tissues and body fluids examined, bradykinin and its close relatives are strong candidates as mediators of inflammation. This reasonable concept has been difficult to substantiate because of the unstable nature of these peptides, which are rapidly degraded by peptidases, and because the kallikrein-kinin system is over-easily activated by a variety of noxious chemical and physical stimuli.[19] However 30 years ago, direct evidence, using a skin perfusion technique, was obtained of the involvement of bradykinin in vasodilation due to local sweating.[20] The only skin disorders in which convincing evidence of involvement of bradykinin exists are the complement C_1 esterase deficiency of hereditary angioedema and in angioedema and urticaria as an adverse reaction to the angiotensin converting enzyme inhibitors captopril and enalapril.[21,22]

Neuropeptides

As long ago as 1901, Bayliss[23] showed that stimulation of the posterior ramus of the dorsal root ganglion evoked skin vasodilation in the innervated skin. Lewis[5] also believed that neural pathways, in the form of the axon reflex flare, were important in the vasodilator response to painful stimuli. Neuropeptides identified histochemically in skin in association with sensory nerve terminals include substance P, vasoactive intestinal peptide (VIP), somatostatin and calcitonin gene related peptide (CGRP).[24–26] Substance P, VIP and CGRP are all highly potent vasodilators on human skin.[27-29] Antidromic depolarization of sensory nerves causes release of SP from the epithelial nerve endings.[30] Furthermore, topical application of capsaicin, the active principle of chili, which depletes SP from sensory nerve endings, causes inhibition of the axon reflex flare, a pathway known to involve antidromic depolarization.[31] The importance of antidromic vasodilation in the inflammatory response to injury is uncertain, and studies need to be carried out on sensory denervated skin. The possibility that centrally initiated antidromic depolarization results in cutaneous co-release of SP, CGRP and other vasoactive neuropeptides, is interesting, and could have important implications in the pathogenesis of relapses of atopic eczema, psoriasis and 'dry' vasomotor flushing attacks, in which psychological factors are implicated.

PHARMACOLOGICALLY ACTIVE LIPIDS

The realization in the 1960s that amines such as histamine and serotonin and the highly vasoactive short chain peptides, including bradykinin, could not by themselves account for the pathophysiological events occurring in clinical inflammation, prompted a search for alternative mediators. Furthermore, no mediators recognized at that time could account for a key component of the inflammatory response, leukocytic immigration into the injured tissue, a phenomenon to which Metchnikoff had many years previously attached such importance. The discovery of a new class of highly pharmacologically active fatty acids, the prostaglandins in the 1960s initially seemed to provide an alternative explanation for the molecular events occurring in inflammatory responses.

Prostaglandins

Prostaglandins were first identified by Von Euler[32] in 1935 in semen and thought, wrongly, to derive from the prostate gland. In fact, the seminal vesicles were the source of seminal prostaglandins which were later purified and structurally identified in the 1960s by Bergstrom and Samuelsson.[33] The next major event was the finding that non-steroid anti-inflammatory drugs (NSAIDs), including aspirin and indomethacin, inhibited cyclo-oxygenase, the enzyme responsible for transformation of the parent essential fatty acid arachidonic acid to prostaglandins, in therapeutic concentrations.[34] It is now widely believed that aspirin and related NSAIDs owe their analgesic and antipyretic activity to their ability to suppress prostaglandin synthesis. That human skin contains prostaglandin synthetase has been demonstrated by radiochemical methods.[35] In homogenates of skin from six donors the mean total prostaglandin activity measured was 1·38 μg/g wet weight/40 min, of which 50% was PGE_2 and 49·5% $PGF_{2\alpha}$. Although initially there was great excitement about the possible role of prostaglandins, it appears that, although synthesized in a wide range of inflammatory dermatoses, they have only a limited role as mediators. This seems most clear cut in erythema due to ultraviolet B (UVB) irradiation. By use of a suction bulla method, exudate was obtained at different time intervals from skin irradiated with three minimum erythema doses (MED) of UVB.[36] Consistently elevated levels of prostaglandins E_2 and D_2 were found, the time course showing concordance with the onset of development and regression of UVB erythema. Furthermore, both the redness and the elevated prostaglandin levels could be partially suppressed by administration of indomethacin.[37] Although prostaglandin D_2, the major eicosanoid product of human skin mast cells, is co-released with histamine in physical urticarias, quantitative considerations argue against an important role for this prostaglandin in urticaria.

Leukotrienes and related hydroxy fatty acids

Apart from the cyclo-oxygenase pathway, arachidonic acid can also be transformed enzymatically via lipoxygenase pathways. Leukotriene B_4 (LTB_4), a powerful pro-inflammatory agent, is formed by the 5-lipoxygenase enzyme which also catalyses the formation of the peptidolipid leukotrienes, C_4, D_4 and E_4. A 12-lipoxygenase enzyme leads to the formation of 12-hydroxy 5,8,10,14-eicosatetraenoic acid (12-HETE) which is also a potent neutrophil chemoattractant. The neutrophil chemoattractant properties of these fatty acids renders them especially attractive as candidate mediators. We found that topical application of LTB_4 in nanogram amounts to intact human skin causes an inflammatory reaction associated histologically with neutrophil polymorphonuclear microabscess formation within the epidermis.[38]

It is, therefore, of special interest that elevated levels of LTB_4, 12-HETE and the parent compound arachidonic acid are present in lesional skin of patients with chronic plaque psoriasis compared with non-lesional skin.[39,40] Elevated concentrations of LTB_4 have also been reported in allergic contact dermatitis (but not in primary irritant dermatitis)[41] and in eosinophilic cellulitis.[42] The major source of LTB_4 in psoriatic skin is uncertain, although LTB_4 production by human mixed epidermal cell suspensions has been demonstrated.[43] Persuasive though the evidence for lipoxygenase products as inflammatory mediators in psoriasis initially appeared to be, confirmation of their role has proved elusive, especially when measured against the requirements of the criteria of Dale.[44] Systemically and topically administered anti-psoriatic agents have been demonstrated to cause both amelioration of the clinical signs of psoriasis and concurrent reduction in eicosanoid levels, but in none of these studies could the possibility that the observed pharmacological effects are secondary to the clinical actions be excluded.[45,46] However, recently, lonapalene, a selective 5-lipoxygenase inhibitor effective in the treatment of chronic plaque psoriasis, has been demonstrated to lower levels of LTB_4 in lesional skin as early as 3 days after topical application,[47] and the possibility that lipoxygenase inhibitors may be of value as an adjunctive treatment in the management of chronic plaque psoriasis and other inflammatory dermatoses needs to be pursued.

Platelet activating factor (PAF)

Recently, attention has been directed towards a distinct phospholipid degradation product, PAF. PAF is an ether phospholipid produced by the action of phospholipase A_2 on ether-linked glycerylphosphoryl cholines via the 2-hydroxy metabolite, lyso-PAF. PAF is powerfully pro-inflammatory in human skin in picomole doses,[48] causing leukocyte infiltration histologically; but the immediate component of the biphasic vascular response appears to be independent of leukocyte involvement or histamine release. PAF has recently been demonstrated in scale extracts from scale of lesional skin in patients with psoriasis, its identity being confirmed by gas chromatography mass spectrometry.[49] It is of especial interest that PAF and prostaglandin E_2 act synergistically to produce wealing.[50] Possibly, PAF may act synergistically with PGE_2 in psoriasis and other inflammatory dermatoses. Both are produced as a result of activation of phospolipase A_2.

Complement C_{5a} and C_{5a} des arg

The complement system is widely distributed in the tissues and plasma. Both C_{5a} and its stable derivative C_{5a} *des arg* are powerfully pro-inflammatory in skin.[51] Their ability to cause increased vascular permeability is not due to a direct local action, but to their potent neutrophil chemoattractant properties. PGE_2, a potent cutaneous vasodilator, greatly potentiates the pro-inflammatory actions of C_{5a} by increasing the blood supply to the tissue. Recently, C_{5a} *des arg* has been identified in scales from patients with chronic plaque psoriasis.[52] No C_{5a} was detected. C_{5a}, generated by C_5 convertase, undergoes desargination by carboxypeptidase N. Although C_{5a} des arg is less potent that C_{5a} as a neutrophil chemoattractant, the large amounts present in the scale of psoriatics may betoken an important role in the pathogenesis of the inflammatory processes involved in this disorder, especially in a synergistic relationship to other mediators.

Interleukin-1 (IL-1) and related cytokines

A cytokine similar to, or identical with, IL-1 is produced by cultured human epidermal cells and has been termed ETAF (epidermal cell derived thymocyte activating factor).[53] Recombinant IL-1 is strongly pro-inflammatory in human skin, causing redness, oedema and a mixed leukocyte dermal infiltrate upon intradermal injection in human skin in picomolar dosage.[54] In animals, injection of ETAF causes fever, a neutrophil leukocytosis and elevation of acute phase proteins. Like IL-1, ETAF is assayed by its activity in the mouse thymocyte co-stimulator bioassay. IL-1-like activity has been found in large amounts in normal skin including heel callus[55,56] and in psoriatic scale.[57] Recently, IL-1-like activity recovered from psoriatic scale has been resolved by further chromatographic purification into at least five distinct peptide components with molecular weights within the range 10–30 kd, all of which are distinct from IL-1 and have neutrophil chemoattractant properties.[58] It appears that the neutrophil chemoattractant activity originally ascribed to ETAF may have been due to contamination by a related peptide or peptides of similar molecular weight and chromatographic characteristics.

The role of IL-1-like activity in psoriasis and other inflammatory dermatoses remains to be established, but recent reports of the effectiveness of the immunosuppressive drug cyclosporin A in psoriasis[59] strengthens the view that T lymphocyte-mediated immune mechanisms may be involved in the pathogenesis of psoriasis. It is also of interest that elevated levels of IL-1-like activity have recently been recovered from UVB-irradiated human skin.[60]

CONCLUSION

Delineation of the role of the several classes of candidate mediators of inflammation in human skin will have to await the acquisition of selective antagonists and inhibitors. Meanwhile, it can be safely assumed that for most inflammatory dermatoses, no single mediator is responsible for the observed pathophysiological changes, and switching off one mediator pathway is unlikely to do more than cause a temporary imbalance in a system of interlocking pharmacological processes. This is hardly a novel notion. In 1927, Lewis[5] pointed out: 'To maintain, for the moment, the reservation that histamine is still not finally proved to be the substance liberated in tissue injury, especially to meet a possibility that more than one substance is sometimes concerned . . . I shall speak of H-substance . . . '. Search for potentially useful new anti-inflammatory drugs should most usefully be directed towards inhibition of 'upstream' molecular events common to several specific 'downstream' mediator cascades, or suppression of target cellular events common to the actions of a range of mediators, such as leukocyte chemotaxis and adherence or vascular endothelial cell permeability.

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Centenary Review

Urticaria: then and now

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It is tempting to think that dermatology has emerged from the doldrums only in the past 30 years. No doubt the editor of this Journal, in his preface to volume 1 in 1888, was thinking just the same when justifying a new journal for a progressive discipline. We cannot but be humble at the present state of urticaria, great though some advances have been. Not very much earlier, Hebra[1] had indicated that urticaria was of no great importance because it seldom killed people. Nevertheless, the Journal did have 41 papers on urticaria in the first 12 years of its life in the nineteenth century. Physicians of that time, mirrored in the text books of Hebra, Radcliffe Crocker (1888)[2] and McCall Anderson (1887)[3] were well aware of the diversity of causes and of the fact that they failed to find any cause in most cases. Hebra scoffed at such theories as electricity in the air, but who would have dreamed of vibration angioedema? Papular urticaria was still *the* urticaria of childhood and at that time only tenuously equated with insect bites.

For a common disease, it is not surprising that urticaria can be recognized in the writings of Hippocrates, Pliny and Celsus. The word urticaria was apparently first used in 1771. Willan brought some order to the classification of skin diseases, but perhaps it did not help the understanding of urticaria so much for it to be classified into six groups on the basis of its appearances and given a binomial botanical-type terminology following Linnaeus. However, it was an advance on Sydenham who grouped urticaria with erysipelas. It was not until the mid-nineteenth century that physicians started to take an interest in mechanisms of disease, and even in the first volume of this journal an author felt constrained to write that it is important to think about mechanisms. Radcliffe Crocker still adopted what was largely Willan's classification.

The term angio-neurotic oedema deserves a special mention. The concept of neurosis implied that the vasomotor nerves supplying the small blood vessels performed their function in some abnormal way. That they were involved in some way was considered 'beyond question'. There was no implication of any effect of the mind on the skin.

The early history of urticaria has been well reviewed.[4,5] We may well smile at some of the old theories and can muse with interest on what will make people smile in another 100 years. The literature on urticaria since 1888 in voluminous. This review will highlight only a few selected or recent topics of interest. Fuller details may be found in monographs on urticaria.[5–8]

MECHANISMS

To have any reasonable understanding of any case of urticaria we need to know (a) the mediators involved, (b) any external or internal trigger, and (c) why those mediators are released in that patient in response to that stimulus. Virtually never are we able to answer all those questions to our own and our patients' satisfaction. We are used to the multifactorial nature of, for example, hand eczema or atopic dermatitis. Perhaps we need to be more aware of multiple causes acting together or sequentially in urticaria. The overlap between the various types of physical urticaria is commonplace. What about the occasional underlying drug allergy or worm infestation with dermographism, or a food allergy and pressure urticaria? What about the patients who only get their attacks of urticaria, perhaps associated with the symptoms of anaphylaxis, when certain foods are combined with exercise; neither exercise nor the food alone being capable of causing symptoms?

Conventional allergy

The physicians of the 19th century were well aware of the numerous causes of urticaria which we would now attribute to an IgE mediated allergy. An early issue of the Journal described how injection of hydatid fluid into the skin would produce a weal only in the (sensitized) patient, but not in a control;[9] also that food given to a wet nurse might transmit urticaria to a neonate. The theory that a simple allergy must be the main cause of urticaria has surely done more to cloud the understanding of urticaria than to clarify it, although, of course, the huge amount of research into the broader aspects of atopic disorders has contributed so much (see p. 437 this issue). These days, dermatologists are constantly confronted by patients wanting an allergy work-up and are well aware that traces of formalin in cavity walls or in cavitied teeth can cause trouble, along with 300 or so other substances that have caused reactions far rarer than some of the more obscure genodermatoses. Anaphylactic sensitivity to the powder in rubber gloves warns us to watch for the obscure.[10] Would that there were a simple screening test that could at least say that a particular urticaria was allergic in origin irrespective of cause.

Immune complexes. The role of immune complexes, usually IgG, has attracted much attention in the past 20 years and quite rightly so. They seem to be responsible for just as many cases presenting to dermatologists in this country as are caused by the prototype IgE allergies. Unfortunately, there seems to be no clear cut-off, using either clinical, histological or other laboratory criteria. There seems to be a complete gradation between florid urticarial vasculitis and ordinary idiopathic urticaria.[11,12]

Delayed allergic reactions. These have attracted much interest recently, especially with regard to asthma. Clearly their understanding is also important for urticaria. Not many years ago, delayed reactions 6–12 h after skin testing were attributed to an Arthus reaction, whereas an IgE mediated component is now recognized.[13,14] A recent paper has emphasized the presence—not necessarily the significance—of such reactions in delayed pressure urticaria.[15]

Non-allergic urticaria

The term pathergy had much to recommend it between the wars, at least as a cloak for, or admission of, ignorance. A recent multi-volume monograph on pseudo-allergic reactions (PAR) shows that there are still questions to be answered.[16] Such mechanisms are convincingly held responsible for the hypersensitivity to aspirin shown by up to 50% of chronic urticaria patients.[17] The evidence for a similar reaction to tartrazine and other food additives is still controversial.[18,19] There has been a gradual fall away in medical interest for these as a cause of urticaria, counterbalancing an increasing popular awareness. So many patients now talk of the dreaded E numbers, all to be tarred with the same brush and with no awareness that E implies approval by the European Community. Currently, sulphites are attracting attention as causes of sometimes very dramatic hypersensitivity (probably not allergic) reactions that may include urticaria.[20]

The literature on all aspects of contact urticaria has expanded hugely in the last 10 years.[21,22] Many of the examples cause few diagnostic or even clinical problems. It is of interest that histamine seems to play little part in some of these non-allergic reactions and that aspirin may be a better inhibitor than antihistamines.[23]

Mediators

The mast cell was only 10 years old when the first edition of this Journal was born, but received regular attention in the early issues. It is still an act of faith to believe that it has a central role as the source of mediators in urticaria. The bewildering numbers of potential mediators rises with every decade so that there is now an *embarras de richesse*,[5,7,24] (see also p. 419 this issue). Only now is evidence accruing that at times there may be more mast cells in patients with urticaria, although it is not known whether this is a primary or a secondary phenomenon. Recent work has repeatedly shown that agents which release histamine from mast cells do provoke larger skin reactions in urticaria patients than in controls.[25,26] The releasability of histamine from blood cells, notably basophils, has given varied results.[27-29] Surely these must be promising ways forward. Little work seems to have been directed at the not uncommon familial, but non-atopic, cases.

The unravelling of the aetiology of hereditary angioedema has been very satisfactory and is still proceeding.[5,7,30,31] Finding of the genetic deficiency of C_1 esterase inhibitor has led to an understanding of acquired examples associated with abnormal lymphoma proteins or with autoantibodies specifically directed against the C_1 esterase inhibitor but with no lymphoma.[32,33] Other complement deficiencies may contribute to other syndromes.[34] It is well established that such inhibitors as antitrypsin, anti-chymotrypsin and others may be abnormal in chronic urticaria, but so far there is no very close clinical correlation.[35,36] Other serum abnormalities have been reported recently.[37] In general, the understanding of the mediators in physical urticarias is better because such reactions can be reproduced at will.

Histology

It might have been expected that the last word could have been said by Unna, who did indeed study urticaria. Only recently has there been a resurgence of interest. Unequivocal urticarial vasculitis clearly has a characteristic histology, but with subtle nuances verging on those of ordinary urticaria.[11] Frank vasculitis is found in only a small proportion of cases of clinically idiopathic urticaria.[11,12] Here the main finding is a monocytic cell infiltrate made up predominantly of T4 suppressor cells.[38,39] The finding of a polymorphonuclear infiltrate in some cases is perhaps surprisingly linked more with the physical urticarias[40] but, so far, helps little in establishing a diagnosis.

CLINICAL CLASSIFICATION

The following rather daunting table (Table 1) lists a large number of distinct 'clinical entities', sadly identified mainly by trigger mechanisms. The table also includes the actual numbers (and percentages) seen in our group of hospitals over a period of 30 years. These were all cases referred to the dermatology department, covering a population of about half a million. Such cases are highly selected, in that only perhaps 5% of cases are referred to hospital in this area. All cases of urticaria are included except those that were clearly a drug reaction or due to insect stings or bites. These 2310 cases represent the largest comparable group so far published, and include the 554 cases already published in 1969.[41,42] They do not include those numerous cases

TABLE 1. A clinical classification of urticaria showing the numbers of cases seen in one dermatology department in 32 years

		Cases seen	
		No	%
Physical urticarias			
Dermographism		199	9
Delayed dermographism		1	0·4
Pressure (see also below)		12	0·5
Cold—idiopathic		44	2
reflex		2	0·1
familial		0	—
cryoprotein		0	—
cold agglutinin		0	—
Heat—local		4	0·2
Solar—about 4 types		9	0·4
Vibratory angioedema		2	0·1
Aquagenic urticaria		6	0·3
Aquagenic pruritus		10	0·4
Mixed physical urticarias			
Dermographism + cholinergic		8	0·3
Dermographism + pressure		2	0·1
Dermographism + cold		2	0·1
Dermographism + idiopathic		19	0·7
Pressure + idiopathic		47	2
Cold + pressure		1	0·04
Cold + cholinergic		2	0·1
	Total	370	16
Cholinergic urticaria			
Cholinergic urticaria		81	3·5
Cholinergic + idiopathic		3	0·1
Cholinergic angioedema		2	0·1
Cholinergic pruritus		2	0·1
Adrenergic urticaria		0	—
	Total	88	3·8
Hereditary angioedema			
Ordinary		10	0·4
Variant		2	0·1
Acquired		0	—
	Total	12	0·5
Histamine releasing agents			
Systemic		0	—
Contact		2	0·1
	Total	2	0·1

TABLE 1 (*contd*)

	Cases seen	
	No	%
IgE allergic		
Ingested foods	51	2·2
Inhaled	8	0·3
Contact	20	0·9
(Injected drugs and stings excluded)		
Total	79	3·4
Urticarial vasculitis/IgG	48	2·1
(Partial forms excluded, but *c.* 3–5% of idiopathic)		
Infections, including *Candida*	24	1
Idiopathic		
Ordinary	1650	71
Micropapular	5	0·2
Bullous	2	0·1
Total	1657	72
Clinical entities defined by associated symptoms		
Urticaria, fever, eosinophilia	1	0·04
Urticaria and vomiting	1	0·04
Urticaria/asthma/anaphylaxis		
food	0	—
exercise	3	0·2
exercise + food	2	0·1
idiopathic	7	0·3
cholinergic—see above		
drug and insect excluded		
Pregnancy	3	0·2
'Auto-immune progesterone urticaria'	3	0·2
Melkersson-Rosenthal syndrome	3	0·2
Muckle-Wells syndrome (1 family)	3	0·2
Familial Mediterranean fever	0	—
Still's disease	0	—
Cocarde purpura (neonatal haemorrhagic oedema)	4	0·2
Total	30	1
Overall total	2310	approx. 100%

TABLE 2. Some factors which may aggravate idiopathic chronic urticaria

Aspirin and related compounds
Foods
Food additives
Systemic illness, including hormonal changes
Focal sepsis
Candida
Psychological

of atopic dermatitis and other atopic disorders in whom a history of allergic urticaria emerges *en passant*. The fact that some 70% of all cases remain idiopathic can allow no complacency. Some factors aggravating idiopathic urticaria are listed in Table 2. So often, such patients react to more than one stimulus, and removal of all apparent stimuli fails to achieve a cure. For this reason such cases are classified as idiopathic where others might list them elsewhere.

CLINICAL ASSESSMENT

Today, what should a bewildered physician do, when confronted with a bewildered patient, that he would not have done 100 years ago, knowing that such a huge multiplicity of factors have at times been reported to have upset so many mediators by mechanisms so often obscure? The author has recently been through the records of the 2310 cases tabulated above, with particular emphasis on 800 cases seen personally in the past 17 years, together with the further 554 reported earlier.[41] It is assumed that the regular evaluation of such patients includes:

History:	Duration of weals
	Pattern of attacks
	Provoking factors, including drugs
	Associated symptoms
Examination:	
Investigation:	Full blood count, ESR (usually normal)
	Follow up any lead from the history
Management:	Food diary in relation to attacks
	Food additive free diet for 3–4 weeks (or longer depending on frequency of attacks)

Although more needs to be done for research purposes, what is missed for routine clinical practice that might be picked up by doing a comprehensive series of tests? Many of these patients have been followed up repeatedly and many investigations performed, others have been seen less often but would have been expected to return to the hospital had they developed, for example, thyrotoxicosis or lupus erythematosus.

Occult allergy to some everyday substances

No doubt cases of this are missed, or suspected and never proven. Only one patient with resistant chronic urticaria later discovered that he was sensitive to coffee and completely cured himself by avoiding it, with repeated relapses after re-exposure. The role of dietary penicillin is still uncertain.[43] It seems to be the experience of generations of clinicians that even the most strict dieting fails to cure more than the occasional case of chronic urticaria. Inhaled causes are rare except in patients who are clearly atopic.

Immune complex urticaria

The gradation between urticarial vasculitis and idiopathic urticaria means that it is possible to miss some cases who have for example raised circulating immune complexes but no suggestive clinical symptoms. It is our experience that such cases are best managed as idiopathic urticaria without the specific remedies available for urticarial vasculitis.[12]

Systemic disease

Not one of our cases of idiopathic urticaria subsequently developed systemic lupus erythematosus (SLE). However, there were three patients with clinically typical urticarial vasculitis who were found to have SLE. A relationship between urticaria and thyroid disease may exist, not necessarily with active thyrotoxicosis.[44] In only two of our cases was there any evidence of thyroid disease, even on prolonged follow-up, and in both of these the thyroid problem was apparent at the time of presentation with urticaria.

C_1 esterase inhibitor deficiency

The clinical diagnosis of hereditary angioedema is usually straightforward. It would seem to be a complete waste of resources to undertake this estimation where the clinical picture is that of idiopathic urticaria, with or without angioedema. Even cases presenting with angioedema alone usually have other clinical markers if it is due to C_1 esterase inhibitor deficiency.

Worm and other infestations

The myth that intestinal worms are a common cause of urticaria seems at last to have died. Even when worms are found, their elimination does not seem to lead to cure. The occasional case of hydatid disease can cause urticaria or even anaphylaxis. Liver fluke infestations have been associated with dermographism.[45] In the absence of any other symptoms these seem rare enough that a blood eosinophilia can be used as a reasonable, if not totally reliable, screen. Scabies may apparently present as urticaria.

Candida

This is more important. Undoubtedly there are cases of idiopathic urticaria in which *Candida* sensitivity can play a contributory part.[46] It could be argued that an intradermal skin test with *Candida* antigen should be part of the routine management, but, in the author's experience, this has yielded a low number of convincing successes. There can also be the occasional case where there is a comparable relationship with a *Trichophyton* infection.

Psychological causes

A link between these and urticaria has been implicated for a very long time and they may be one factor in 15–30% of cases. This is usually very apparent during routine history-taking. Deep seated psychological causes have been held responsible, but searching for these tends to be of little avail in the hands of most clinicians.[47]

Rare variants of urticaria

These are not likely to masquerade as idiopathic urticaria. They are mostly defined by associated symptoms which are unlikely to be missed on even casual history-taking.

The general message must be that, for routine clinical management, investigation is largely inappropriate unless there is a good lead from the history. It is an expensive form of placebo if just to reassure the patient that no stone is being left unturned. Time is better spent on history-taking, explanation and more careful attention to details of drug therapy. Even the value of a blood count and ESR might be challenged! The painstaking hours spent by the Shelleys[48,49] can but be admired, but in the follow-up of the 1300 cases studied here personally, virtually nothing emerged that had not been at least suspected at the first interview. We need to look for multiple causes, but not to jump to conclusions, in a disease which is so unpredictable and with such a strong tendency to spontaneous remission.

THERAPY

Looking at the treatments of 100 years ago, it is no wonder that quacks were so abundant in the eighteenth and nineteenth centuries, and earlier. Conventional medicine had virtually nothing to offer apart from toxicity—emetics; the inevitable purges; aloes, belladonna and nux vomica pill; arsenic; quinine; potassium bromide; sulphuric acid and even perchloride of mercury. The treatment section of Joltrain's monograph on urticaria of 50 years ago reads almost as strangely, although there are perhaps some acceptable pharmacological reasons why autohaemotherapy and autourotherapy might work.[50]

Antihistamines may not be the entirely logical treatment for a disease or diseases in which histamine may or may not play a key role, but over 70% of urticaria patients are prepared to admit some benefit from their use. The occasional case of urticaria actually aggravated or caused by antihistamines should not be forgotten. H2 blockers seem to have a limited role.[51] There are many special drugs and treatments for special variants—dapsone, danazol, colchicine, tranexamic acid, plasmapheresis and even aspirin and non-steroidal anti-inflammatory drugs. Inhibitors of the other mediators seem to be just round the corner, not only as useful therapy, but also to throw light on basic mechanisms. The excitement grows!

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Centenary Review

Atopy: one hundred years of antibodies, mast cells and lymphocytes

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When the first volume of the British Journal of Dermatology was published (1888–1889) there was some awareness of the clinical manifestations of allergy. Magendi[1] (1839) had reported that dogs re-injected with egg albumin died suddenly, and Blackley[2] (1873) wrote graphic descriptions of injection and inhalation of pollen in a hay fever subject, (himself), but the concepts and terminology were unknown. The term 'anaphylaxis' was introduced by Portier and Richet (1902), 'allergy', by von Pirquet (1906) and 'atopy' and 'reagins' by Coca and Grove (1925), although reagin had been referred to earlier by Citron in 1908, the *British Journal of Dermatology* was slow to participate in the developing knowledge. The term anaphylaxis first appeared in the index in 1916, allergy in 1926, atopy in 1941, antigen in 1943, and antibody in 1958, although reference was made to lymphocytes forming antibody in 1954. All refer to abstracts.

Antibodies, reagins and IgE

An early interest in antibodies was their use as antitoxins. However, the therapeutic use of untreated horse serum or globulin inducing classical serum sickness, made clinicians aware of the side effects, later known as allergy, and the distinction between the immediate anaphylactic signs and those mediated by immune complexes. Investigation of spontaneous anaphylactic allergies was thwarted by failure to detect antibodies by conventional techniques, until the famous report by Prausnitz and Küstner[3] (1921), in which serum from Küstner, who was allergic to fish, was injected into the skin of Prausnitz, and 24 h later the site was challenged with fish antigen, resulting in a weal and flare with itching. A similar phenomenon had been induced earlier, unwittingly, by Ramirez in 1919, when a patient given a blood transfusion from a donor very sensitive to horse dander, developed bronchial asthma 2 weeks later on two occasions when exposed to horses. Both the recipient and the donor gave positive skin reactions to great dilutions of horse dander antigen.

From observations of clinical signs, interest developed in histamine as a mediator of anaphylaxis, first in tests on actively sensitized tissue, skin *in vitro* (1942), blood cells[4] (1941), and lung *in vitro*[5] (1951), followed by *in vitro* passive sensitization of blood cells, of lung[6] (1967) and of skin[7] (1972). It was the Prausnitz Küstner (PK) test and the *in vitro* passive sensitization tests that enabled examination of the properties of the reaginic antibody and its eventual identification as IgE.

Rapidly improving chemical and physical fractionation procedures were used to separate and characterise the different immunoglobulin classes. Reagin, for a short time, was thought to be IgA, but this was rejected when pure anti-IgA did not remove reaginic activity from serum.

As in several instances in the history of atopy, a major new finding was reported almost coincidentally by two laboratories. Painstaking critical fractionation of allergic sera led eventually to the identification by Ishizaka *et al.*[8,9] of a new immunoglobulin IgE as the reagin. In Sweden, Johansson *et al.*[10] found a myeloma protein designated IgND which was found to be immunochemically identical with IgE.[11] The properties of IgE, its presence in trace amounts in sera, ability to sensitize human skin anaphylactically, ability to block sensitization by reaginic sera, and the susceptibility to heat of the sensitizing activity, were all consistent with its identification as the reagin. With increasing availability of samples, no subclasses of IgE were found, but in amino acid sequence analysis two domains were identified which vary in heat susceptibility. It has now (1988) been possible to map the sequence of amino acids between two domains, Cε2 and Cε3, of human IgE which, when folded together, probably form the Fc component binding to mast cell IgE receptors.[12]

Apart from *in vitro* cell sensitization tests, assay of the trace amounts of IgE would have remained difficult, had not the larger amounts of the myeloma IgND expedited the preparation of pure anti-IgE and the development of an assay method for total IgE (1966), and the now well standardized radio-allergosorbent test (RAST) for antigen-specific IgE antibodies.[13] It could also be detected by antiglobulin red cell agglutination tests[14] which prepared the way for detection of cell-bound IgE by several methods. Assays of serum IgE antibodies, and detection of IgE on mast cells, basophils and in tissue became routine procedures.

The concentration of serum total IgE is usually related to the type of atopic disorder and its severity. In allergic rhinitis, and allergic acute urticaria the concentrations may be within the range found in normal non-atopic persons, but increasing amounts occur in asthma, atopic dermatitis, and greatest of all in atopic dermatitis concomitant with another atopic disorder, e.g. asthma. However, the concentrations of total or antigen-specific IgE are not absolute indications of atopic disorders; in some allergic persons little may be detectable, and in some clinically normal persons considerable amounts of specific IgE are present.

Human IgG as an anaphylactic antibody

The classical anaphylactic antibody of man is IgE. However, there is evidence that in some human sera, there is an IgG anaphylactic antibody or component contained in IgG uncontaminated by other immunoglobulins, including IgE, that confers antigen-specific anaphylactic sensitivity on skin.[15] The IgG antibody differs from IgE in that it binds weakly to human skin in PK or monkey PCA, sensitizing the sites for 2 to 4 hours only; the weal and flare is less intense than an IgE response, but is equally susceptible to antihistamine treatment; the ability to sensitize tissue is unaffected by heat or by treatment with a chemical reducing agent, it binds weakly to tissue or basophils *in vitro*, and is easily eluted by washing. It will block passive sensitization of monkey skin by IgE antibody depending upon the relative concentrations of each.

The identity of the antibody has not yet been determined, and it was designated IgG S-TS (short-term sensitizing).[15] It has been claimed that IgG_4 has similar sensitizing activity, but although IgG_4 is found in increased amounts in sera of patients with atopic dermatitis and other atopic disorders, and some samples of IgG_4 will block sensitization of monkey skin by IgE for a short time, there is no certain evidence that IgG_4 anaphylactically sensitizes human tissues.

Mast cells, basophils and their anaphylactic mediators

The first descriptions of mast cells and basophils are attributed to Ehrlich (1878, 1879), but mast cells in unstained frog connective tissue were described 15 years earlier by Recklinghausen (1863). These were undoubtedly connective tissue mast cells, but, as early as 1905, Maximow identified 'atypical' mast cells in rat mucosa, which are now designated mucosal mast cells. The function of mast cells was long controversial, and it was contended that they provided reserves of tissue ground substance, later identified as sulphated mucopolysaccharides and hyaluronic acid, or, by releasing heparin, prevented blood coagulation. Their origin was also obscure, although, possibly fortuitously, mast cells were seen to develop from lymphocytes by Downey (1911) and to be associated by Riley with fibroplasia and derived from blood vessel adventitia. The relevance to atopy became apparent in the 1950s when Riley[16] demonstrated that release of histamine by chemical agents was closely associated with disruption of mast cells. Apart from an abstract (Stuart 1952) reporting disruption of mast cells in anaphylaxis, the first detailed study of anaphylactically-sensitized mast cells degranulating on challenge with antigen was made in 1959.[17] The term mast cell first appeared in the index of the British Journal of Dermatology in 1905, and basophil degranulation, by paper title only, in 1964.

Studies on mast cells made slow progress until their content of histamine and relationship to anaphylaxis stimulated interest in their IgE-binding properties and content of pharmacological mediators. Purified IgE and anti-IgE were essential reagents to identify and isolate IgE receptors (FcεR) on animal mast cells or human basophils. There appears to be an equilibrium between the numbers of FcεR on human basophils and amounts of serum IgE, the numbers of receptors increasing with IgE concentration. The FcεR from human basophils has been isolated by IgE-Sepharose, but on rat mast cells, subjected to different treatment, there is evidence of at least two receptors,[18] one with strong, the other with weak affinity for rat IgE. However, of particular interest is the fact that the weak receptor also binds IgG. This may be analogous to the binding of IgG S-TS and IgG_4 to human basophils, with transient blocking or sensitization by IgE.[15] Currently, several laboratories are analysing the chemical structure of the IgE receptors.

The cross-linking by antigen of IgE on the membranes of mast cells and basophils activates several membrane enzymes, resulting in the secretory (non-cytotoxic) release of preformed mediators, mainly from the granules, and generation of others, mainly derived from lipids. The first mediators associated with anaphylaxis were histamine from human mast cells and basophils, and serotonin together with histamine from rodent mast cell granules. As interest increased and most clinical signs and histological changes of anaphylactic allergy were attributed to mast cell substances, several other mediators were isolated, those of most immediate relevance being the pre-formed chemotactic substances, some preferentially attracting eosinophils, others neutrophils, and the freshly generated prostaglandins, leukotrienes, and platelet-activating factor.

Probably the most significant recent advance in knowledge of mediators is the recognition that slow reacting substance (SRS or SRS-A of anaphylaxis) that stimulates a slow sustained contraction of smooth muscle *in vitro* not inhibited by antihistamines, and released from several types of cells following allergic or inflammatory stimuli, e.g. actively sensitized anaphylactic guinea-pig lung,[19] passively sensitized anaphylactic human lung[6] and human basophils, was a complex of substances designated leukotrienes (LT).

In 1980, three laboratories, in Sweden, the UK and the USA, each reported on the lipoxygenase products of arachidonic acid. SRS was found to represent at least three leukotrienes,[20] LTC_4, and its conversion products LTD_4 and LTE_4. These, together with

LTB_4, derived first from neutrophils and reported a year earlier, proved to mediate many of the changes in anaphylactic disorders, to be unstable and of short half-life *in vivo*, but very potent, LTD_4 being 1000-fold more potent than histamine in contracting smooth muscle.

Investigations on the origins of mast cells also reveal increasing complexity. There are at least two, and probably three, distinct cell types, (1) the connective tissue, typical or serosal mast cells, (2) the mucosal, atypical or intestinal mast cells and (3) the globule leukocytes which may be derived from the mucosal cells or represent a distinct cell line. Although the connective tissue and mucosal cells differ morphologically, and in their content of mediators, both have receptors for IgE and degranulate on antigenic binding. However, there are strong indications that, in both laboratory animals and man, there is considerable heterogeneity between mast cells in different tissues, e.g. lung and skin, and in their susceptibility to therapeutic agents.

Present evidence supports the view that whether or not there is a common precursor cell, connective tissue mast cells are derived from fibroblasts, without T cell regulation (as seen in cultures of mouse embryonic skin), and mouse mucosal mast cells originate from lymph node cells grown in the presence of T cells or a T cell factor derived from antigen-challenged sensitized T lymphocytes.[21] Similarly, rat mucosal cells have been obtained from cultured thymocytes[22] and are thus T cell dependent even if not of direct T cell origin.

Lymphocyte abnormalities in atopy and atopic dermatitis

The relevance of changes in lymphocyte activity as a fundamental feature of atopy is little understood. Although cells resembling lymphocytes were described by van Leeuwenhoek, it was not until interest in: (1) tuberculosis in the early 1900s, (2) evidence of delayed hypersensitivity to protein antigens in 1929, and (3) the far-reaching studies of Medawar and his colleagues in the 1950s on skin graft rejection, that critical interest in lymphocyte function was stimulated. The *British Journal of Dermatology* reflects this trend, in that histological descriptions of cells, undoubtedly lymphocytes, were presented in 1892 and in many later papers. However, the first index reference to lymphocytes was in 1954 in relation to antibody formation (abstract) and lymphocytes in skin (1957). From 1965 onwards there was an interest in lymphocyte transformation tests in the detection of allergic sensitization. The first paper with T lymphocyte in the title appeared in 1976.

The recognition that lymphocytes were a diverse cell population, prepared the way for studies on function. The separation of human lymphocytes into those with intrinsic bound surface immunoglobulin[23] and those that made receptors for sheep red blood cells,[24] which were becoming known as T and B cells on the basis of animal studies,[25] was rapidly confirmed, and T cells further divided into subsets expressing receptors for Fc IgG (T_G or T_γ) and for Fc IgM (T_M or T_μ) that were related to function.

T_γ cells suppressed and T_μ cells helped or stimulated B cell antibody synthesis *in vitro*.[26] This was followed closely by tests on mouse thymus and spleen cells, revealing the Ia or immune antigen on T cells with Fc receptors, thus linking the ability of T cells to initiate an immune response with properties of regulating the nature and intensity of the response; all achieved between 1970 and 1976. More critical differentiation of lymphocyte subsets was possible when lymphocyte epitope-specific monoclonal antibodies became available.

Even before the identification of helper and suppressor cells, Rajka[27,28] had repeatedly reported that atopic dermatitis was associated with reduced delayed hypersensitivity responsiveness, subsequently confirmed, and further shown that there tended to be a decreased total number of T cells, a definite decrease in T_γ suppressor cells, and decreased responsiveness *in* *vitro* to antigens and mitogens. Furthermore, there are several reports that the numbers of natural killer (NK) cells (antibody independent cytotoxic lymphocytes) are decreased or that they are ineffective.

Recent investigations have demonstrated that T cells from atopic dermatitis patients are unable to suppress B cell synthesis of IgE *in vitro* unless stimulated by the mitogen Con A; whereas T cells from non-atopic persons effectively reduced B cell IgE synthesis, providing evidence of the failure of T suppressor systems in atopy.

Further recent evidence of T cell dysfunction in atopic dermatitis is the inability of the cells to suppress B cell synthesis of IgE *in vitro* unless stimulated by the mitogen Con A, whereas T cells from non-atopic persons effectively reduced B cell IgE synthesis.[29] Other possible immunological defects reported in atopic dermatitis are: (1) the poor ability of Langerhans cells to stimulate proliferation of autologous T cells, which is interpreted to indicate reduced formation of T cells responsible for suppression of IgE synthesis, and (2) reduced synthesis of an epidermal cell interleukin-1 (IL-1) factor that regulates lymphocyte proliferation on antigenic stimulation.[30]

The detection of lymphocyte IgE receptors (FcεR) offered another approach to examine lymphocyte regulation of IgE synthesis and deviations from normal in atopy. Lymphocyte FcεR differ from those of basophils in having a weak affinity for monomeric IgE, being non-reactive to anti-basophil FcεR antibody, and appearing on a very small proportion of peripheral blood lymphocytes, in contrast to their presence on all basophils. The percentage of cells with FcεR usually increases with the concentration of serum IgE, and although most of the lymphocytes are B cells, approximately 0·1% T cells express FcεR in atopic persons, and have been identified as cytotoxic/suppressor and killer/natural killer subsets.[31]

The significance of these findings is still being investigated, but it is evident that atopy, and atopic dermatitis in particular, is a manifestation of reduced immunocompetence with a selective deficiency of T cell suppressor activity, which in turn may be a manifestation of impaired endogenous mediator synthesis, thymus hormones and interleukins.

Atopic disorder therapy in the future

The achievements in the last 30 years since the observation of anaphylactic degranulation of mast cells, and 20 years since the identification of IgE, in the studies of the fundamental processes of immune responsiveness and atopy, have been remarkable. Nevertheless, these achievements seem to have contributed little to therapy. Antihistamines were in use before the relevance of mast cells was understood. Altounyan (1967) did not require knowledge of IgE or of mast cell mediators to find the valuable therapeutic inhibitory activity of disodium cromoglycate, nor have others in developing beta-blockers and related drugs.

Although the search for chemical inhibitors to control clinical signs continues, it is likely that the results of the immunological studies will have therapeutic applications. One probability is the preparation of very small molecular weight antigenic epitopes that will bind with and block IgE on mast cells without cross-linking to initiate mediator release. Another is treatment with thymus hormones or interleukins to induce maturation of T cells and correct deficiencies in their regulatory activities.

These measures could correct some atopic disorders, but severe immune deficiencies resulting in atopic dermatitis, intractable asthma, or combinations of atopic allergies, may well require a more fundamental approach. When the genetic coding of human chromosomes has been achieved, and the genes controlling immune regulation identified, it is conceivable that analysis of foetal cells will reveal the potential defects long before birth and eventually, DNA genetic sequences could be injected into the foetus to supplant the defective genes. For this genetic prophylaxis to be acceptable, society will have to reconsider its moral and ethical standards.

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Centenary Review

Psoriasis

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'Psoriasis is not only amenable to treatment, but curable'.

The above quotation is taken from the first report on psoriasis published in the first volume of the *British Journal of Dermatology* 100 years ago.[1] Unfortunately, time has shown that this statement was not true then, and it is still not true in 1988.

The first recognizable description of psoriasis is attributed to Celsus (25 BC–45 AD) in his '*De re medica*', nearly 2000 years ago. The disease was described under the heading of impetigo (from the Latin word *impeto*, to attack). Galen (133–200 AD) is credited as the first person to use the word psoriasis, but he was probably describing seborrhoeic eczema—'Scaling of the eyelids and corners of the eyes with great itching and much excoriation'. However, Galen used the word correctly as it comes from the Greek word 'psora' the itch. Hippocrates probably did see psoriasis, as he grouped scaly eruptions under the term lopoi (from lepo, to scale). Thus, from the outset, it could be argued, the wrong names were given to clinical diseases we recognize today. Up until the eighteenth century, psoriasis was grouped with leprosy, and psoriatics often suffered the same fate as lepers, including being burnt at the stake in the fourteenth century. In 1809 Willan was the first to accurately describe psoriasis and its various manifestations but he did not separate it with certainty from leprosy. This was left to Hebra in 1841.

Thus, 100 years ago, psoriasis was recognized as a definite clinical entity, the histology had been described, the terms acanthosis and parakeratosis introduced by Auspitz (1835–1886), who also described the characteristic bleeding points when the scale is removed in psoriasis—Auspitz's sign.'

One of the most significant clinical observations made in psoriasis (the Koebner phenomenon) had already been described in 1872. In addition, in volume 1 of the *British Journal of Dermatology* there was also an excellent account 'Psoriasis and joint afflictions'[2] describing many of the features of psoriatic arthropathy that we recognize today.

CLINICAL ASPECTS

In Thomas Bateman's book in 1819 he refers to Willan's classification of psoriasis as guttata (guttate), diffusa (plaque), palmaria (palmar) and inveterate (chronic with thick scales). During the nineteenth century the majority of the clinical patterns of psoriasis became established as we recognize them today. At the turn of the century in Radcliffe Crocker's third edition of *Diseases of the Skin*[3] the following clinical variations were described. P. punctata which are small pinhead size flat papules which enlarge to about $\frac{1}{4}$ inch and appear 'like drops of water' on the skin, then referred to as P. guttata. As the lesions continue to enlarge they become P. nummularis or discoidea. The coalescence of several patches covering the greater part of a limb or the trunk is

P. diffusa and when all over the body P. universalis. Involution of the disease always commences at the centre of the plaque giving rise to a ring of psoriasis, P. circinata. Persistent and obstinate psoriasis where the skin is thickened and fissured, and has adherent scales, was termed P. inveterata, and where the scales were raised in conical heaps, P. rupoids. Finally, P. acuta was recognized in which the disease appeared as bright red patches, with less well defined margins, the scales were thin and papery, being thrown off rapidly with no time to accumulate; the skin was hot and tender and itched severely. This suggests the inflammatory component as being an important part of the disease process, and the more severe the inflammation the worse the disease.

It was also recognized that the site of the disease may modify the appearance, e.g. the scalp in which hair loss may occur, on the palms where crusts are seldom found but fissures are common, and on the genitalia where there is less scaling but redness, induration and some secretion.

Linear psoriasis or P. striata had been described, and it was known that involvement of the mucous membranes was very rare. Nail involvement either alone or associated with disease elsewhere had been recorded and included pits, discolouration and thickening.

Thus, most of the clinical features of psoriasis were well established by the turn of the century. However, the history of the so-called pustular forms of psoriasis has not been so clear cut and there is still argument today concerning the nomenclature and whether some of the forms are in fact psoriasis. The history of pustular psoriasis began in 1888 with the description of a condition by Radcliffe Crocker termed dermatitis repens (weeping), which was a persistent vesicular eruption of the extremities and considered to be due to bacterial infection. In 1890 Hallopeau described 'acrodermatitis supperativa continua' being a persistent pustular eruption on the digits. Generalized pustular psoriasis was described in 1910 by von Zumbusch and its relationship to psoriasis is not disputed. The peripheral pustular eruptions were considered to be the same disease by Barber and Eyre,[4] but Ingram[5] considered that there were two distinct pustular eruptions of the extremities, one (and the generalized pustular eruption) were associated with staphylococcal infections, and he suggested the term acrodermatitis perstans, however, the acrodermatitis of Hallopeau which had negative bacteriology should be called pustular psoriasis. In the same edition of the *British Journal of Dermatology*, Barber[6] suggested the term acrodermatitis perstans for the condition dermatitis repens (Crocker) and for the acrodermatitis of Hallopeau, but the term pustular psoriasis to describe an eruption consisting of discrete red patches with pustules on the palms and soles. However, not everyone agrees that the term pustular psoriasis for this particular eruption is correct, as they dispute that it is part of the clinical expression of psoriasis, and consider it a separate entity. In the last 60 years the following terms have appeared in the literature to describe the localized eruption on the palms and soles: pustular psoriasis of the extremities, pustular bacteride, pustulosis palmo-plantaris, acrodermatitis pustulosa perstans, intractable eruptions of the hands and feet, pustulosis palmaris et plantaris and relapsing pustular eruptions of the hands and feet. This is an example of descriptive dermatology excelling itself (characteristic of the first half of this century), often serving to confuse and adding nothing to the understanding of the nature of the problem. The evidence that localized pustular psoriasis (of the palms and soles) is part of psoriasis comes mainly from the relatively high incidence of plaque psoriasis in the disease, reported as 10–25%. However, in the clinically uninvolved skin in localized pustular psoriasis there is no increase in DNA synthesis and mitotic counts, as found in patients with psoriasis,[7] and the incidence of HLA antigens B13 and B17 is not raised in this entity.

The relationship of acrodermatitis continua (the pustular eruption of the digits around the nails) to psoriasis has also to be determined. Undoubtedly, in the elderly it may evolve into generalized pustular psoriasis and there are also reports of its association with plaque psoriasis. Acrodermatitis continua is a rare form of psoriasis, the incidence being as low as 0·4% of all psoriasis. Until we have better ways to investigate the genetic and pathogenetic mechanisms in skin diseases, the relationship of psoriasis to pustular psoriasis, acrodermatitis continua, and generalized pustular psoriasis remains to be determined. It may well be that all these clinical entities have a common genetic basis, but that there are modifying genetic and environmental factors which influence expression of the disease.

Age of onset

One hundred years ago, standard texts on dermatology stated that psoriasis was rare under the age of 3 and that in two-thirds of patients the disease began before the age of 30. Radcliffe Crocker (1903) reported only 22% of cases beginning between the ages 30 and 50 and only 5·5% over the age of 50, although it may present in the ninth decade. These statements would not be out of place in a current textbook in 1988.

Sex ratio

The fact that psoriasis tends to affect males and females equally was already well established 100 years ago.

Prognosis

It is unlikely that reports in the literature give a true indication of the overall severity of the disease and remission and relapse rates. All the studies apart from Lomholt's[8] are from preselected groups of patients attending a specialist clinic. Patients with minor involvement or disease of short duration may never consult a doctor or arrive in hospital clinics. Thus, probably, most studies will tend to give an over pessimistic view of the natural history.

One hundred years ago the prognosis was reported as follows: 'Overall the prognosis for the disease is bad. Although the disease is amenable to treatment it is not possible to prevent recurrences which are almost certain to occur in at least 90% of patients. The frequency of recurrence is variable. In some patients the disease is persistent, in others, attacks are an annual event whilst in others, the interval between may be years. Left to itself it may go on for many years with remissions and exacerbations, or it may sometimes disappear spontaneously'. These comments are indeed accurate, and subsequent studies during the last 100 years, although supplying specific figures, have only tended to confirm what was known then.

One of the first significant attempts to study the course of psoriasis was that of Romanus.[9] Of an original 1417 patients seen between 1892 and 1922, 54% were available for follow-up over a mean period of 21 years. In these patients 13% were free of the disease, 16% had intermittent episodes, and 71% had continuous skin lesions. In Lomholt's study of 30,000 inhabitants of the Faroe Isles, similar figures were obtained.[8] Over a 20-year period only 6% were completely disease free, whereas at 10 and 5 year follow-up, 14% and 17% were disease free, respectively. However, Lomholt found during a 20-year period that 48% of patients would have a remission at some time. Conversely, 48% would have an exacerbation of their illness. Farber and Nall[10] in a study of 5355 patients reported that 39% had a remission of their disease, in 29% of whom it was spontaneous. The duration of remission varied from 1–54 years. Thus, psoriasis runs a chronic course, interspersed with remissions in approximately 40–50% of patients, but Radcliffe Crocker[3] appears to have been correct when he said only 10% of patients can expect to have a permanent remission. Lomholt in his comprehensive study looked for possible prognostic indicators, and he concluded that early onset, i.e. under the age of 10, implied severe and persistent disease, and patients with later onset i.e. over the age of 25, tended to have mild disease. Psoriasis commencing on the knees, elbows and scalp had a better prognosis than disease which began on the face and trunk. The prognosis did not differ in patients who had a parent with the disease and those who did not. Erythrodermic and generalized pustular psoriasis are now generally accepted as having a poor prognosis. Although guttate psoriasis is self-limiting in the majority of patients, 68% of these patients subsequently had chronic plaque disease.[11] Pustular psoriasis on the palms and soles is well recognized to be persistent and one study found only 28% of patients had cleared after 10 years of the eruption.[12]

AETIOLOGICAL FACTORS

Many of the aetiological factors in psoriasis that we recognize today were known 100 years ago. However, although we may, as yet, not be able to fully explain how these factors actually produce the skin lesions in biological terms, they surely provide the clues that we need to unravel the pathogenetic pathway. Too often, hypotheses put forward to try to explain psoriasis do not take into account the known precipitating and aggravating factors.

The Koebner phenomenon

In 1872 Heinrich Koebner delivered a lecture to the Silesian Society for National Culture entitled 'The aetiology of psoriasis'. In this talk, Koebner described how in a patient with psoriasis, excoriations from riding, suppuration from a co-existing lymphadenitis, a horse-fly bite and a tattoo had been followed by an outbreak of psoriasis limited to the damaged skin area. Koebner considered that these observations refuted earlier ideas on the aetiology namely 'dyscrasia' and 'embolic genesis'. He considered that there was a 'peculiar disposition located in the skin of psoriatic patients that remains latent for years at a time, but produces this chronic form of inflammation of the skin under the influence of a great many internal and local stimuli'. From this viewpoint Koebner considered that the sites of predilection for the first appearance of the rash, the temporary nature of the success that follows the local destruction of individual lesions, and the great tendency of the disease to recur, were all explained. He concluded that 'therapy must not be merely symptomatic, but designed to reduce the vulnerability of the whole skin'. This shows a remarkable understanding of the nature of the disease, and that was 116 years ago.

Since psoriasis does not occur, and cannot be induced, in animals, the Koebner phenomenon is the only experimental model that investigators have to study factors which may induce psoriasis and the subsequent changes in the skin. During the early part of this century investigators who studied the Koebner phenomenon were of the opinion that the primary event was vascular and, therefore, dermal. However, more detailed and recent studies showed that epidermal damage had to occur to produce the Koebner reaction, and dermal damage alone did not do so.[13,14]

Observations on the Koebner phenomenon have also implicated central or humoral factors in the control of the disease. It is generally agreed from most studies that only 25% of a randomly selected group of patients with psoriasis are Koebner positive. This positivity is an all or none phenomenon, i.e. if the patients are positive at one site, the same reaction will occur at another site at the same point in time. However, patients may vary at different times in whether they are Koebner positive or negative. In 1982 Eyre and Krueger[15] introduced the term 'the reverse Koebner reaction' in which an area of plaque psoriasis that was removed, healed to give clinically uninvolved skin. Patients who exhibited the reverse Koebner reaction were always

Koebner negative and it was mutually exclusive with Koebner positivity. These observations imply a central controlling factor which varies from time to time and influences the response to injury in patients with psoriasis. The nature of this factor is unknown, but it could be locally produced, such as a T suppressor cell factor. In support of this suggestion is the observation by Eyre and Krueger that patients who were Koebner negative have a milder course on follow-up, and resolution of psoriasis has been shown to be associated with an increase of activated T suppressor cells in the psoriatic lesion.[16] In addition, it was recently shown that Koebner positive patients have a higher T helper/suppressor ratio in uninvolved skin compared with Koebner negative patients.[17] However, this still begs the question as to what determines this ratio, whether it is a central factor, or a local one such as concentration of antigen. Whatever controls the Koebner response, it does not appear to be associated with extent or clinical pattern of the disease. However, a correlation between age of onset and Koebner response has been reported, in that of patients whose disease began before the age of 15, 75% were Koebner positive, whilst in those whose disease began after 30, only 5% were positive.

Infections

At the end of the last century one of the theories as to the cause of psoriasis was infection. Micrococci had been observed on the surface of psoriatic lesions and implicated. However, to explain the symmetry of the eruption it was argued that the organism must gain entry to the blood stream and then be disseminated. In addition, specific infections had also been implicated in the last century, including pleurisy, influenza, scarlet fever, erysipelas and smallpox. In the first half of the century infection was often mentioned as a precipitating factor, but no specific infection was linked to psoriasis. In 1938, one report on a series of 170 patients mentioned tonsillitis being present in 3% before the first attack of psoriasis, and in 5%, further bouts of psoriasis appeared after tonsillitis. In 1947, Norrlind reported that 54% of 66 patients with psoriasis had a positive streptococcal agglutination test compared with 7% of patients with venereal disease and 26% of patients with various other dermatoses including infections. Three years later the same author reported in a selected series of 32 patients, 27 of whom had guttate psoriasis, that 18 patients had raised anti-streptolysin titres. About a week before the eruption 16 patients had had a throat infection, in 11 haemolytic streptococci were found in the fauces, and 15 of 18 patients with guttate psoriasis had a raised ASO titre.[18] Similar results implicating streptococcal infections in guttate psoriasis were also reported by Norholm-Pedersen in 1952[19] who found 44% of 133 patients had a history of infections precipitating the eruption, and in three-quarters of these the streptococcus was implicated. Infection as a precipitating factor was commoner in young people and usually resulted in guttate psoriasis.

It is now generally accepted that streptococcal infection is the major precipitating factor in guttate psoriasis. How the streptococcal infection causes psoriasis is still under debate (as it is for rheumatic fever and nephritis). However, a recent study[20] with streptococcal monoclonal antibodies has shown binding to components of keratinocytes. Thus, if there is cross-reactivity between streptococcal antigens and keratinocyte components, this could explain the initiation of a disease process. There is accumulating evidence from other disorders that infections may precipitate disease in genetically predisposed individuals, giving rise to a self-perpetuating auto-immune state. It is possible that a similar situation exists in psoriasis, and it is known that genetic factors play an important role in the disease. However, it still has to be explained why guttate psoriasis is self-limiting in the majority of patients compared with the persistence of chronic plaque disease. The obvious possibilities are that there are different triggers, or that there are different genetic factors influencing clearance of the antigens or response to these antigens. The role of other infective agents, particularly viruses, has yet to be determined in psoriasis, but they may well prove to be important.

Heredity

At the end of the last century, heredity was considered an important aetiological factor in psoriasis. It had also been stated that psoriasis may skip generations and that it was rare for all the family to be affected. Most of the reports in the first half of the twentieth century, based on family studies of large series of patients, agreed that heredity was important and the mode of inheritance was dominant, but irregular. Hoede, working in Wurzburg, has made a significant contribution to the genetics of psoriasis with details of patients and families being recorded since 1925. In his early reports, Hoede[21] found a family history of the disease in 34% of patients, in 1931 this had increased to 39%, and by 1957 to 56%. The general consensus is that approximately one-third of patients have a positive family history, but the incidence obviously depends on so many variable factors that it is not surprising that widely differing results have been reported in the literature. However, there were dissenters concerning the hereditary nature of psoriasis, notably Schamberg (1924) who claimed there was no proof of heredity, and Siemens who stated in 1929 that 'the only thing we know with certainty about psoriasis is that we know nothing definite about it'. Studies in the 1950s and 1960s of families with direct inheritance through three and four generations strongly supported the concept of Medelian dominance with incomplete penetration, as every affected child had an affected parent. However, another study of six generations did not find that all individuals with psoriasis had an affected parent and asymptomatic carriers were assumed.[22] These latter workers stated that the inheritance was simple autosomal dominant with 60% penetration, but Watson *et al.* in 1972[23] considered inheritance to be multifactorial, implying that the cause of the disease is due to the effect of several genes, each with a small effect, with a permissive role being played by one, or possibly a few, major genes.

Major contributions to a heriditary role in psoriasis were made by the census studies of Lomholt[8] and Hellgren.[24] Lomholt's census was of a third of the 30,000 inhabitants of the Faroe Isles, and Hellgren's was of 40,000 individuals in parts of Sweden. Hellgren found that 6·4% of relatives of psoriatics were affected, compared with 1·96% of the general population. Lomholt found a familial occurence in 91% of the cases, but this was in a closed community of the Faroe Isles. Subsequent analysis of Hellgren's and Lomholt's data was compatible with the current concept of multifactorial inheritance, and it is now thought that simple monogenic types of inheritance have been excluded in psoriasis.

Studies on twins also strongly support the role of hereditary factors in psoriasis, concordance in monozygotic twins being approximately 65–70% and that in dizygotic twins 15–20%. Bandrup in his studies on twins also found that the clinical type of psoriasis, age of onset, the course and severity, was determined to a large extent by genetic factors.[25] This implies that the genetic make-up may be even more important than previously assumed.

During the 1970s, techniques for detecting histocompatibility (HLA) antigens were developed. These antigens are inheritable and the corresponding chromosome region lies on the short arm of chromosome 6. The genes in the HLA region code for cell membrane components of importance in the immune response. It is now thought likely that the HLA genes are not themselves responsible for the disease, but that the actual disease genes lie near the HLA complex on chromosome 6. However, there is thought to be an association between the HLA genes and the disease genes, so that determining the HLA status gives a good indication of the risk of the disease.

Early studies on psoriasis showed an association with the class I antigens B13 and B17, and subsequently B37, and genetic linkage has been shown to exist between these antigens. However, the association with these antigens, although significant, is considerably weaker than in many other diseases with HLA association e.g. ankylosing spondylitis, dermatitis herpetiformis and type I diabetes mellitus. More recently, it has been proposed that the primary association in psoriasis is with CW6 and that B13, B17 and B37 are secondarily associated. The latest reported association is with the class II antigen DR7 and it may well be that there are two susceptibility genes, one associated with CW6 and the other with DR7. It should be emphasized that not all patients have these disease markers and there is variation between populations.

HLA studies on varying clinical patterns of psoriasis have shown that genetic factors may well be important in the different clinical expressions. Patients with only flexural psoriasis do not have an increased frequency of B13 and B17, but B13 is associated with early onset of the disease and streptococcal infection-related exacerbations. The incidence of B13, B17 and B37 is normal in patients with localized pustular psoriasis on the palms and soles, in acrodermatitis continua, and in generalized pustular psoriasis. HLA antigen studies in psoriatic arthropathy have shown correlations with the disease. If sacroiliitis is present there is a significant association with B27, and A26, B38 and DR4 have all been reported to be increased in psoriatic arthropathy in general.

There has long been debate about the role of genetic factors and the modifying role of environmental factors in psoriasis. It is possible that genetic factors play the dominant role. Identification of disease susceptibility genes is now in its infancy, but with new techniques the genes responsible for psoriasis will eventually be known and reveal the varying genetic combinations that determine the clinical expression of the disease.

Other precipitating factors

Stress has been known to precipitate and to aggravate psoriasis since the last century. However, the nature of stress and how it influences the biological events that precipitate disease is not well understood in the 1980s. Hormones obviously play a fundamental role in the organic manifestations of stress and they are, therefore, likely to influence the pathogenesis of diseases such as psoriasis. There is now accumulating evidence that immunological processes are also affected by stress. Other than accepting that stress may influence the psoriatic process, we shall have to await the exact scientific explanation.

Certain drugs, notably lithium, β-blockers, indomethacin and anti-malarials have been reported to aggravate the disease. However, considering the common use of these drugs it is possible that the aggravation of the disease may be an idiosyncratic response, as it certainly does not occur in all patients with psoriasis taking the drugs. As these drugs all have different modes of action, all that can be said is that they are likely to be influencing the psoriatic process at different points in the complex biochemical pathways involved in the disease.

Hypocalcaemia has been reported to aggravate psoriasis, but since it is in itself an extremely rare condition, it plays little role in the aetiology of the disease. However, the observation should be remembered as it may tell us something about the pathogenesis of the disease, particularly as vitamin D has been reported as benefitting psoriasis.

PATHOGENESIS

One hundred years ago the pathology of psoriasis had already been adequately described and the arguments for the primary histological event were well under way. Unna, in his textbook *Histopathology of Skin Diseases* in 1896, emphasised parakeratosis, white cells in the prickle cell layer, epithelial growth and dilation of blood vessels as being important features. He was undecided as to whether the inflammation or epithelial growth was the primary event but considered that they may both be part of the same process which produces psoriasis. Unna mentioned the micrococci in the horny layer as a possible initiating factor and related it to induction of psoriasis as seen in the Koebner phenomenon—Koebner himself mentioned a suppurating wound as one of the precipitating factors in psoriasis. Auspitz considered the lengthening of the dermal papillae as due to epithelial downgrowth and not to papillary growth into the epithelium, and he considered the changes in the blood vessels as due to chemotactic factors which dilate and then lengthen the blood vessels. He also described a cellular infiltrate in the neighbourhood of the vessels, consisting of mast cells, a few plasma cells and connective tissue cells. Considering that Auspitz died in 1886, his observations show a remarkable understanding of the pathological processes in psoriasis which are not out of place today but seem to have been ignored by many research workers in the twentieth century. Radcliffe Crocker, in 1896, sums up the concepts of psoriasis as follows: 'There are two theories as to how psoriasis commences (1) inflammation in the dermis is the primary event and hyperplasia secondary, (2) inflammatory changes in the dermis are a secondary event.' He added 'neither theory throws light on the nature of the pathological process or what starts the process and of that we are quite ignorant.' Some may say that we have not advanced from that statement over the last 100 years.

In the first half of the twentieth century, there appear to have been no significant advances in our understanding of the pathogenesis of the disease. In their paper 'Histopathologic study of psoriasis' in 1943, Burks and Montgomery[26] mention very few new observations, although they did comment on the numerous mitotic figures in the basal and prickle cell layer and the fact that mitotic figures were rare in the epidermis after 2 weeks treatment with the Goeckerman regimen. A significant study of the hyperplasia of psoriasis was reported in 1963 by van Scott and Ekel.[27] Carrying out mitotic counts, they reported a mitotic count of 50·4 in a 2 mm length of psoriatic skin compared with 1·86 in uninvolved skin, and calculated the turnover time of psoriatic epidermis as 4 days compared with 27 days for normal epidermis. Van Scott and Ekel did not come to any conclusion as to the initiating factor for the hyperplasia in psoriasis, but considered it was secondary to changes in the dermal papillae. The studies by van Scott and Ekel led to the assumption that the other epidermal characteristics of psoriasis were all secondary to the increased mitosis of the keratinocytes, and from this followed the hypothesis that psoriasis was uncontrolled cellular proliferation of the epidermal cells. Van Scott and Ekel's work also led to another assumption, that antimitotic drugs are bound to be helpful in the management of psoriasis as they suppress the 'primary fault'. These assumptions, that psoriasis is due to uncontrolled mitosis and that drugs work in psoriasis by their antimitotic effect, are commonplace in textbooks of dermatology in the 1980s. However, they ignore and do not fit with certain observations. Firstly, in wound healing, in experimentally induced hyperplasia and in certain other skin disorders (e.g. bullous ichthyosiform erythroderma) the mitotic counts are the same as that seen in psoriasis, and yet psoriasis does not develop. Therefore, the increased mitosis per se cannot account for the histological features of psoriasis. In addition, Fry and McMinn in 1968,[28] in studying the histological and cellular changes in the clearing of psoriasis with chemotherapeutic agents, reported reformation of a granular layer before a fall in mitosis. These results implied that, firstly, increased mitosis was unlikely to be the primary event in psoriasis, and, secondly, that drugs effective in clearing psoriasis did not have their primary antipsoriatic effect on mitosis, but on some other part of the psoriatic process. Despite these observations, during the late 1960s and 1970s the principal force of research into the nature of psoriasis was directed at the increased mitosis and proliferation of the epidermis, as this was considered by many as the primary inherent fault in the disease. If there was increased mitosis and a shortened transit time for the epidermal cells, the cell cycle was likely to be altered. This was first investigated by Weinstein and Frost in 1968[29] who reported the cell cycle as being reduced to 37·5 h compared with 457 h for normal epidermis. Since then, there have been many other estimates of the cell cycle time in psoriasis, most showing a longer time than that obtained by Weinstein and Frost. However, more recently, it has been questioned whether the cell cycle is altered in psoriasis, as the techniques that were used are fallacious, and it is now proposed that the hyperplasia is due to an increase in the number of germinative cells in psoriasis. As recently as the early 1980s a symposium was held on 'Psoriasis: cell proliferation' which was justified as important in both the pathogenesis and treatment of the disease.[30] This was questionable because of the existing evidence that the proliferation may only be a secondary event and that current drugs used for psoriasis probably did not have a primary action on cell proliferation. It could be said that Unna and Auspitz were more aware of the inflammatory changes and their importance in relation to the psoriatic process than were the researchers of the 1970s.

During the last decade, however, attention has been focussing again on the inflammatory reaction in psoriasis and its possible significance. This task has been made possible by newer techniques, particularly the development of monoclonal antibodies. In the 1970s a number of observations were made implying a possible cell-mediated immunological abnormality in psoriasis. These included depressed reactivity to contact allergens and their correction with treatment, and delayed reactivity to intradermal challenge with antigen, and *in vitro* studies showed depressed response to mitogens, and depressed lymphokine production in response to antigen challenge. There were conflicting reports as to whether the numbers of circulating T lymphocytes were decreased or normal. In the late 1970s the nature of the dermal cell infiltrate in psoriasis was investigated again, and in the early lesions was reported as predominantly T lymphocytes, macrophages and monocytes, but polymorphonuclear cells were not present.[31] Support for a role of the T lymphocyte in psoriasis is now gathering pace in the 1980s. In 1984 Baker *et al.*[32] showed that in patients with extensive disease there was a decrease of both total T cells and T helper cells in the blood, and the T helper/suppressor ratio was greater in established lesions compared with that in the blood. These results were taken to imply a recruitment of T helper cells into psoriatic lesions. In a subsequent study in the same year, these investigators[16] reported that in early guttate psoriasis there was an influx into and activation of T helper cells in the epidermis, and resolution of guttate psoriasis was associated with an influx and activation of T suppressor cells and disappearance of the activated T helper cells. These activated lymphocytes were found in close apposition to the dendritic processes of the Langerhans (antigen presenting) cells (LC) in the epidermis. To implicate the LC further in the pathogenesis of the disease, it was shown that there is an increase in both the total number and the proportion expressing HLA-DR. These observations led this group of workers to postulate that psoriasis is a disorder of keratinocyte proliferation mediated by T lymphocytes.[33] This hypothesis, although not explaining all the known facts, does take them into account. It has long been known that streptococcal infections will precipitate psoriasis. We have recently shown that streptococcal antigens cross-react with cellular components of LC and keratinocytes. Thus, either the streptococcal antigen itself or an auto-antigen may be responsible for initiating the disease. Once the antigen is present in the epidermis, it will be taken up and processed by the LC which will then attract T helper cells into the epidermis and then present antigen to them resulting in their activation. These activated T helper cells will release a number of lymphokines, with known biological effects, which could account for the accompanying histological features of psoriasis, namely, dilated and enlarged capillaries, activated fibroblasts and epidermal proliferation. However, at some stage the hypothesis must take into account the known genetic predisposition in psoriasis. At present, the candidates for this may be an altered response of the psoriatic epidermal keratinocyte to cytokines, a specific susceptibility to respond to certain antigenic stimuli, e.g. viral or bacterial, or a failure in the mechanisms to clear antigen in the disease. In support of the first is the observation that psoriatic keratinocytes respond differently to γ-interferon (IFN) compared with normal keratinocytes.[17] γ-IFN is known to inhibit cell proliferation and the failure to respond to γ-IFN may contribute to the hyperproliferation associated with psoriasis.

The hypothesis proposed by Valdimarsson *et al.*[33] is also compatible with the known mechanisms of action of therapeutic measures in psoriasis, and predicted the response of the disease to one of these, namely cyclosporin. The hypothesis fits with the beneficial effect of UV radiation which is known to reduce the number of functional LC and affect T cell function. It has been shown that both PUVA and topical steroids significantly reduce the number of epidermal T cells prior to clinical and histological improvement. It is more plausible that current therapeutic agents exert their beneficial effect on psoriasis on the cells concerned with immune function in the skin rather than by directly inhibiting processes concerned with cell division of the keratinocytes, although it is accepted that many of our current therapeutic agents for the treatment of psoriasis are likely to have more than one site of action.

In any discussion on the pathogenesis of psoriasis it is important to discuss the clinically uninvolved skin and ask why only certain parts of the skin are affected by the clinical lesions in the majority of patients. Over the last 30 years it has been shown that clinically uninvolved skin is not normal. There is increased DNA synthesis by the epidermal keratinocytes,[34] there are dilated capillaries and there is a dermal lymphocytic infiltrate with activated T helper and T suppressor cells. Lymphokines produced by the activated T cells could explain the other features, but others would prefer to think that the increased DNA synthesis may represent an inherent defect of the epidermal keratinocyte. The observation that there is a dermal lymphocytic infiltrate would be compatible with the potential for all the skin to develop psoriasis, the deciding factor being the amount of antigen(s) present, be it dermal or epidermal.

Biochemical abnormalities

With the advent of new techniques to study intracellular enzymes since the 1950s, there were several reports detailing abnormal findings in psoriasis. Most of these would be accounted for by the increased metabolic activity of the psoriatic keratinocytes, relating to carbohydrate and protein synthesis; none could be said to be specific for psoriasis, and they are probably of a secondary nature.

Any biochemical abnormality that is found has to explain the characteristic hyperproliferation of psoriasis. Voorhees and Duell[35] postulated that a defect in the cAMP cascade was compatible with many of the changes seen in psoriasis. It was argued that there were low levels of cAMP, and this would favour the accumulation of presursors required for cell replication, and hence be associated with hyperproliferation. However, subsequent studies did not confirm low levels of cAMP in psoriasis, and individual enzymes involved in the cAMP cascade have also been found to be normal. In addition, no alteration of cAMP levels has been found in uninvolved skin. Thus, it is unlikely that enzyme systems concerned with cAMP are of a primary nature in the pathogenesis.

cGMP and certain polyamines have also been found to be raised in psoriasis, but it is difficult to assign any specific role to these findings. Proteases and antiproteases have been studied in psoriasis as they appear to have a function in cell proliferation and differentiation. It is known that the protease, plasminogen activator is present in increased amounts in psoriatic scales. Plasminogen activator is concerned with cell proliferation and increases prior to cell division. Inhibition of plasminogen activator is brought about by antiproteases which have been found in skin. It has been argued that psoriasis may be due to a lack either qualitatively or quantitatively of a specific inhibitor.[36] However, the evidence for this altered specific inhibitor is speculative and does not adequately explain the monocytic cellular infiltrate in the epidermis and dermis.

Arachidonic acid and its metabolites

During the last decade, pharmacologists and dermatologists have investigated the conversion of arachidonic acid by cyclo-oxgenase and lipoxygenase pathways in psoriasis, in the hope of defining the mediators which may contribute to the psoriatic process and possible pharmacological agents to reverse it. It has been shown that there is a considerable increase in arachidonic acid and of both 5 and 12 lipoxygenase products leukotriene B_4 (LTB_4) and 12 hydoxyeicosatetraenoic acid (12 HETE). Products of the cyclo-oxygenase pathway, namely the prostaglandins, are, in comparison, only moderately increased.[37] It has been suggested that in psoriasis there is a defect in which the cyclo-oxygenase pathway is inhibited, and that 12 HETE and LTB_4 both contribute to the psoriatic process. The increased quantities of LTB_4 and 12 HETE may be the chemotactic factors for the polymorphonuclear leukocyte (PMNL) infiltrate characteristic of psoriasis. However, LTB_4 does not itself produce psoriasis when applied to or injected into uninvolved psoriatic skin. Failure to produce sufficient prostaglandins, particularly PGE_2, compared with lipoxygenase products, may, however, be relevant to the psoriatic process as they are known to be immunosuppressive and will therefore modulate the probable immunological reaction in psoriasis. Support for this comes from the observation that the drug benoxaprofen, which inhibits the lipoxygenase pathway was beneficial in psoriasis.

Polymorphonuclear cells

There is no dispute that PMNL infiltration of the epidermis, and to a lesser extent the dermis, is a characteristic feature of the established psoriatic lesion. It has even been claimed that psoriasis is due to a defect of the PMNL. However, this would appear to be unlikely as the only site of pathology is the skin and occasionally the joints. In addition, it is now generally agreed that PMNL infiltration is a relatively late feature in the development of psoriasis, compared with mononuclear cell infiltration, acanthosis, and increased mitosis. Chemotactic factors for PMNL are well described in psoriasis but they all appear to be formed late in the disease process, again implying a secondary role of PMNL.

TREATMENT

Thomas Bateman in 1819 suggested the following for psoriasis: mercury, a gentle purge, avoid stimulants, a little cream, oil of almonds, and a mild antiphlogistic. However, 100 years ago the therapy of psoriasis had advanced considerably and Radcliffe Crocker (1893) claimed 'I have never seen a case in which it was not possible to remove the eruption for a time although much perseverance is needed. Treatment takes 3 weeks to 3 months to clear the psoriasis, and the more the patient's effort the quicker the response. There is no known way of preventing recurrences which are almost sure to occur'. These statements would probably be equally applicable 100 years later. One hundred years ago the most effective remedy was arsenic taken orally. It was recorded then that there was a great variation in response between patients. Although arsenic was used in other skin disorders it was most effective in psoriasis. Other oral treatments at this time included turpentine, antimony, cantharides and phosphorus. Local treatments were divided into two categories (1) to remove the scales; with soap, salicylic acid in spirit and alkaline baths, (2) specific remedies, which included coal tar preparations, β-naphthol, thymol, pryrogallic acid, turpentine, ammoniated mercury and chrysarobin.

Chrysarobin (3-methyl dithranol) was the forerunner of dithranol as we know it today. Chrysarobin is obtained from the aroroba tree. It was the active ingredient of Goa powder, imported by the Portugese from Brazil into India via Goa and used for the treatment of ringworm. Following misdiagnosis it was found to be effective for psoriasis, as reported by Balmanno Squire in the *British Medical Journal* in 1876. The side-effects were as well recognized then as they are now and were listed as erythema, burning, itching and swelling of the surrounding skin, and the material dyed the skin, hair, nails and linen. Attempts to lessen these side-effects were employed as they are today..A hundred years ago it was found that using chrysarobin in chloroform was helpful in diminishing untoward effects. Thus, two of the three preparations that are used topically today (coal tar and dithranol) were already used for psoriasis 100 years ago.

General advice given at the end of the last century was avoidance of overwork, sexual excesses, suckling and any drain on the body. Spa treatment was recommended for its relaxing effects and some spas even had arsenic in the water.

X-ray treatment for psoriasis first began in 1900 and was used for well over 50 years. Behçet in 1936[38] commented that it had not resulted in single cure, despite its great value in temporarily removing patches of the disease. X-ray treatment for psoriasis was phased out during the 1940s and 1950s because of the known carcinogenic and other side-effects.

A beneficial effect of sun on skin diseases has been known for over 300 years, and it was also recorded 100 years ago that psoriasis was better in the summer than in winter. Finsen in 1903 first used ultraviolet radiation (UVR) for the treatment of lupus vulgaris, and Alderson in 1923 first reported its beneficial effects in psoriasis.[39] In an attempt to increase the effect of UVR on the skin, Goeckerman investigated the effects of photosensitizers and reported in 1925 that prior application of crude coal tar enhanced the effects of UVR.[40] This treatment is still employed in some centres to the present day; it is being replaced by pleasanter and more effective treatments.

There is no doubt that UVR has a beneficial effect on psoriasis, and patients are well aware of this themselves and take holidays in the sunnier parts of the world. Currently it appears that the optimal wavebands are between 300 and 320 nm from high intensity lamps. However, in the Dead Sea Centre, which is effective in clearing psoriasis, it has been claimed that most of the UVB is filtered out by the aerosol of water vapour above the Dead Sea. The disadvantage of phototherapy from artificial sources is that it takes longer to achieve clearance than with PUVA and other treatments, but there does seem to be a place for home units used under medical supervision.

Dithranol was first synthesized in 1916 and still forms the basis of topical treatment in many centres. Treatment with dithranol was modified in 1953 by Ingram, who recommended using it with UVR to increase its effectiveness.[41] This does impose an extra committment by the patient to attend hospital daily, and some have disputed whether the UVR increases the efficacy of dithranol. The advent of short contact dithranol over the last 10 years has virtually seen the end of the Ingram regimen as an outpatient treatment in most centres. Short contact dithranol therapy is based on the observation that by using 20 times the strength of dithranol normaly used, but only for a few minutes, it is possible to achieve the same concentration of the drug in the epidermis during a 24 h period.

Although Goeckerman investigated photosensitizers for use in the treatment of psoriasis and found coal tar to be the best, a more effective one came into use in the early 1970s. Psoralens, which are extracts of plants, are claimed to have been used in the treatment of vitiligo as long ago as 2000 BC, and have been extensively used for this complaint since the 1920s. However, it is claimed that they were too effective as photosensitizers because with UVR they would cause severe reactions if used for any length of time; but if they are used only with UVA the phototoxic effects are considerably less. Tronnier and Schule in 1973[42] first reported the beneficial effect of topical psoralens and UVA on psoriasis and Parish *et al.*[43] subsequently reported the beneficial effect of oral psoralens and high intensity UVA in the treatment of psoriasis, and it became known as PUVA. This therapy is now a highly effective and established treatment for psoriasis, but there are reservations concerning possible long-term carcinogenic effects.

Corticosteroids came onto the scene in 1950, as ACTH, cortisone and topical hydrocortisone. Both ACTH and cortisone were reported to have no appreciable effects on psoriasis, and topical hydrocortisone was equally ineffective. By alteration of the steroid molecule in the 1950s to counteract the mineralocorticoid effect, prednisolone and triamcinolone were synthesized, and these drugs were shown to be moderately effective when taken orally. However, like all treatments, they were only suppressive to the psoriatic process and the disease recurred on cessation of therapy. Because of the side-effects of long-term corticosteroid treatment and possible rebound when the corticosteroids are stopped, these drugs never gained wide acceptance in the treatment of psoriasis. By further alterations to the steroid molecule, potent topical steroid preparations were introduced in the early 1960s. The two best known were fluocinolone acetonide (Synalar®) and betamethasone 17-valerate (Betnovate®) and they were both effective in treating psoriasis. The clinical potency appeared to correlate well with the vasoconstrictor assay introduced by McKenzie and Stoughton in 1962.[44] The clinical effect of topical steroids was further enhanced by using them under polythene occlusive dressings which increased absorption into the skin 10-fold. However, by the end of the 1960s the side-effects of this treatment were well recognized and it was discontinued. The main problems were associated with the effect on the dermal collagen and fibroblasts leading to thinning of the skin, spontaneous bruising and striae formation. In addition, suppression of the pituitary–adrenal axis occurred if this treatment was applied to large areas of the body. In the 1970s, more potent topical steroids were synthesized, the most notable being clobetasol propionate (Dermovate®). There is a good correlation between the potency of the topical steroid and its antipsoriatic effect, but the side-effects of these very strong topical steroids are those seen with the less potent steroids and polythene occlusive dressings. It is now apparent that topical steroids are not the panacea that was hoped for in the treatment of psoriasis. The side-effects associated with long-term use of potent topical steroids are too great and limit their use. In addition tachyphylaxis occurs with topical steroids when used for psoriasis, so they become less effective if used continuously.

In the late 1940s, the folic acid antagonist, aminopterine was shown to have a beneficial effect in leukaemia. Gubner *et al.* in 1951 commented that folic acid antagonists had similar actions to cortisone because both inhibited connective tissue proliferation and suppressed experimentally induced arthritis. They, therefore, treated patients with rheumatoid arthritis with aminopterine, one of whom had psoriasis.[45] They noted improvement in the psoriasis and an exacerbation when citrovorum factor was given because of side-effects of the aminopterine. Following these observations, Gubner went on to treat patients with psoriasis with aminopterine and found a beneficial effect, indeed the psoriasis improved more than the arthritis. Gubner, therefore, postulated that apart from its effects on connective tissue, aminopterine had effects on epithelial tissues which were even more impressive than those on mesenchymal tissues. As with all effective treatments for psoriasis, the limiting factor was the side-effects. Amethopterine (methotrexate) was one of the next generation of folic acid antagonists and Edmundson and Guy in 1958 reported that methotrexate was as effective in clearing psoriasis as aminopterine and was shown to be less toxic.[46] It was subsequently shown from toxicity studies in animals that the shorter the duration of exposure of the tissues to methotrexate the less toxic the drug. Thus intermittent therapy i.e., weekly, should have become standard practice in the early 1960s but did in fact not do so until the 1970s, when hepatoxicity from methotrexate was recognized as a major problem. In 1971, based on their study of the cell cycle in psoriasis, Weinstein and Frost suggested that methotrexate should be taken every 8 h for a 24 h period once a week.[47] This dosage schedule was introduced, as Weinstein and Frost said that the cell cycle in psoriasis was 37 h and that continual exposure of the cells to methotrexate over this period of time should produce a better therapeutic response. Their argument also assumed that the action of methotrexate in psoriasis was by inhibiting DNA synthesis. In fact it has now been shown that the regimen is no more effective than a once weekly oral dose, that the cell cycle is longer than 37 h, and finally, that methotrexate may well be working by another mechanism. Methotrexate is still used extensively in the treatment of psoriasis but its toxicity remains a limiting factor; the advent of PUVA, cyclosporin and retinoids have also decreased the necessity to use methotrexate.

Following the observation that methotrexate was effective in clearing psoriasis, other ‘cytotoxic’ drugs have been tried, most notably hydroxyurea and razoxane. However, although effective, razoxane had a short history because of the risk of inducing leukaemia. Hydroxyurea is certainly no more effective than methotrexate and considerably more toxic, and is rarely used.

It has been known since the beginning of the century that vitamin A was important for the maintainance of epithelial tissues. It is, therefore, not surprising that it should be tried for many skin disorders, particularly those like psoriasis with abnormal keratinization. However, the dose required to produce any therapeutic effect has always been limited by the side-effects. During the last 20 years derivatives of vitamin A, termed retinoids, have been synthesized, and one, etretinate has been shown to have a therapeutic effect on psoriasis. Etretinate has proved to be an effective drug in generalized pustular psoriasis and the acral pustular form of the disease. It is less effective in chronic plaque disease, but is used with other therapeutic modalities, particularly PUVA to decrease the amount of UVA needed to clear the eruption. Unfortunately, like all useful drugs in psoriasis it is not without side-effects. One of the main problems is that retinoids are teratogenic and etretinate has a very long half-life; patients are advised not to become pregnant for at least 2 years after stopping the drug. In addition it increases blood lipids, there is a small risk of hepatoxicity and it may induce extra-osseous calcification and premature epiphyseal closure. The advent of a newer retinoid, etretin, with a much shorter half-life than etretinate, but with a similar therapeutic effect, looks set to replace etretinate in the treatment of psoriasis.

The other fat soluble vitamin, vitamin D (in the form of vitamin D3) has, over the last 3 years, been reported to have a beneficial effect on psoriasis when used either topically or orally. As with aminopterine, this was a chance finding in a patient taking vitamin D3 for osteoporosis; the patient also had psoriasis and this improved. The earlier reports of beneficial effects will have to be substantiated, but in the long-term vitamin D may well prove too toxic for therapeutic use.

The most recent acquisition to the dermatologist’s armamentarium for psoriasis is cyclosporin. Cyclosporin was shown to have an immunosuppressive effect in 1976 by Borel *et al.*[48] and since then its principal action has been shown to be against activated T helper lymphocytes. It has significantly improved the results of organ transplantation, and more recently has been shown to have a beneficial effect in some autoimmune disorders. The first report of a beneficial effect in psoriasis was in 1979 by Meuller and Hermann, when, like aminopterine, the drug was being used for the treatment of rheumatoid arthritis.[49] However, unlike Gubner with aminopterine, neither Meuller and Hermann nor the dermatological world took these observations any further until the mid 1980s. In the standard British dermatological reference textbook by Rook *et al.*, published in 1986, no reference is made to cyclosporin in the treatment of psoriasis. Following the observations of Baker *et al.*[16,32] that in psoriasis there was an influx and activation of T helper cells in the epidermis, it was proposed that activated T helper cells were important in the pathogenesis of psoriasis, and that if this were so, cyclosporin should be effective in clearing the disease. Indeed it has now been shown to be highly effective.[50] Its use, however, is likely to be limited by its nephrotoxicity and the risk of hypertension.

Thus, if we look back over the last 100 years, only two treatments have lasted the course, namely coal tar and dithranol. All the old systemic treatments including arsenic and mercury, and the newer ones of the last 40 years have serious side-effects, and it is doubtful whether any of our current therapeutic regimens will still be used in another 100 years. The major disappointments are twofold. Firstly, we have no drug or other modality to alter the course of the disease and induce a permanent remission, and secondly, we have no safe remedy to control the disease. Apart from cyclosporin, all our current treatments are based on empirical observations and thus it is perhaps not surprising that our therapeutic advance over the last century has been slow. We are either going to have to be extremely lucky and find the remedy by chance, or more likely newer therapies may emerge as a result of an improved understanding of the pathogenesis of the disease.

CONCLUSIONS

The last 100 years has been disappointing both in our understanding and treatment of psoriasis. The clinical, histological and aetiological concepts well expounded in the last half of the nineteenth century were followed by a relatively barren period in the first half of the twentieth century. Our knowledge of the cellular and biochemical abnormalities in psoriasis has certainly increased over the last 30 years, but we still find difficulty in explaining a coherent sequence of events. The literature is littered with investigators' claims that a particular biochemical or cellular abnormality that they have found to be abnormal in psoriasis is 'the cause' of the disease. It is probable that we shall have to await advances in scientific technology to unravel the secret of psoriasis. If I had to speculate on the nature of psoriasis I would opt for susceptibility genes responsible for two major abnormalities. The first an abnormal immune response to an external antigenic stimulus and possibly subsequently, an auto-antigen, and the second, the abnormal maturation and hyperproliferation of the keratinocytes. Each stage would be susceptible to alteration by modifying genes or environmental factors or both. Whatever the future will reveal, hopefully the claims made in the first volume of the *British Journal of Dermatology* 'that psoriasis is a curable disease', will prove correct in the next 100 years.

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Centenary Review

The Langerhans cell

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INTRODUCTION

Paul Langerhans could hardly have predicted a time when most issues of dermatology journals would contain at least one article on the intriguing cell he described whilst still a medical student in 1868.[1] The history of the Langerhans cell over the past 100 years very much parallels the development of investigative dermatology as a discipline over the same period. Advances, particularly in the field of cellular immunology, have enabled us to build on the impetus provided by the earlier classical anatomical descriptive studies. It has emerged that this cell, which constitutes only about 2% of all epidermal cells, plays a critical role in cutaneous immunological reactions as a result of its antigen presenting properties. We now appreciate that Langerhans cells interact with neighbouring keratinocytes, which secrete a variety of immunoregulatory cytokines, recirculating T lymphoyctes and peripheral lymph nodes, to form an integrated system of skin associated lymphoid tissues (SALT), which acts at the most peripheral outpost of the immune system and mediates cutaneous immunosurveillance.[2–5] Study of the Langerhans cell has never been more topical, with reports that this cell may act as a nidus for infection with the human immunodeficiency virus (HIV-I) and contribute to the cutaneous complications of the acquired immune deficiency syndrome (AIDS).[6] The centenary of the *British Journal of Dermatology*, which coincides with the centenary of Paul Langerhans' death in Madeira at the age of 40 from tuberculous renal failure,[7] provides a fitting occasion to review in broad outline the current status of our knowledge of the biology of this crucial component of the skin-associated immune system.

MORPHOLOGY, ONTOGENY AND DISTRIBUTION

Langerhans originally described the cell which bears his name as a suprabasal dendritic cell in human epidermis which stained with the Cohnheim gold chloride technique.[1] Because this technique also delineates neural tissue, Langerhans cells were for many years erroneously thought to be related to the neuroectoderm. An alternative theory suggested that Langerhans cells might represent effete melanocytes. The ultrastructural features of Langerhans cells, including a lobulated or indented nucleus, a clear cytoplasm with rough endoplasmic reticulum and a well-developed Golgi apparatus, but devoid of tonofilaments, desmosomes or melanosomes, and above all the presence of characteristic cytoplasmic granules (Fig. 1), were first defined in 1961.[8] The granules usually appear in thin sections as rods with a central zipper-like striation, but may sometimes be seen as 'tennis racket'-like structures with an expansion at one

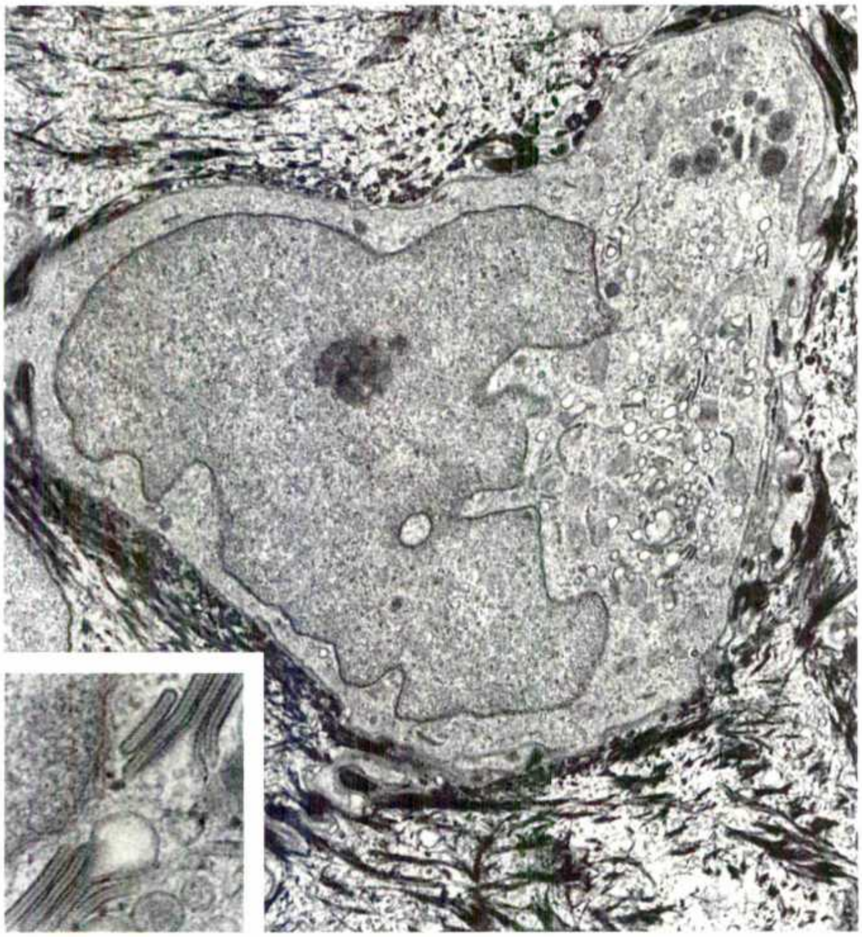

FIGURE 1. Electron micrograph showing ultrastructural appearance of a Langerhans cell (original × 12,500). Inset: characteristic cytoplasmic granules (original × 68,400).

end; three dimensional reconstruction suggests that the granules are disc-shaped with a vesicular bleb at one end. The presence of these characteristic granules has become established as the essential criterion for the identification of Langerhans cells; cells with the general ultrastructural appearance of Langerhans cells, but lacking granules in the section under study are termed 'indeterminate' cells. The function of the granules remains unknown, and opinion is still divided as to their origin from either the Golgi complex or from the cell surface by endocytosis.

Ultrastructural studies did not provide support for a relationship between Langerhans cells and either neuroectoderm or melanocytes; Breathnach *et al.* laid these theories to rest by demonstrating the presence of a full complement of Langerhans cells in murine skin experimentally deprived of its neural crest component.[9] The observation that cells with ultrastructural features identical to cutaneous Langerhans cells are characteristic of lesions of histiocytosis-X[10] prompted speculation that Langerhans cells might represent epidermal histiocytes or macrophages. Using bone marrow chimerization techniques, Katz *et al.*

established in mice that allogeneic donor Langerhans cells first became detectable in the skin of recipients 13 days after chimerization, and that after 85 days up to 80% of the Langerhans cells were derived from the bone marrow of the donor animal.[11] The relatively slow replacement of recipient by donor cells may reflect the longevity of resident epidermal Langerhans cells. Allogeneic Langerhans cells have also been detected in the epidermis of a human recipient of a bone marrow graft.[12] In mice, a second population of bone marrow-derived dendritic epidermal cells, which express surface Thy-1 antigen and are distinct from Langerhans cells, has been described. Murine Thy-1+ dendritic epidermal cells are thought to represent a population of immature T lymphocytes;[13] we and others have been unable to prove the existence of a human analogue for such a cell.[14] The above studies established that epidermal Langerhans cells are derived from and continually repopulated by a mobile pool of precursor cells originating in the bone marrow. In addition, Langerhans cells probably have a limited capacity to undergo proliferation within the epidermis.[2,15]

Langerhans cells are preferentially distributed in mammalian stratified squamous epithelia, including those of the epidermis, oral cavity, oesophagus, nasopharynx, conjunctiva and female genitourinary tract. In man, there is some regional variation in the number of Langerhans cells per unit area of skin, the range being 460 to 1,000/mm^2 of body surface; an average adult man has a total of about 2×10^9 Langerhans cells in his skin.[2] Despite their relatively small number, the dendritic nature of Langerhans cells and their migratory capacity enables them to maintain contact with many keratinocytes, and thus to provide a network capable of surveilling events within the whole epidermis.

SURFACE MARKERS OF LANGERHANS CELLS

The phenotype of epidermal Langerhans cells is quite distinct from that of keratinocytes and of other intra-epidermal dendritic cells (reviewed in Katz, 1985[16]). Langerhans cells exhibit membrane ATPase activity,[17] and, in keeping with their mesenchymal origin, contain vimentin type intermediate filaments, and express surface S-100 protein as well as surface antigens characteristic of cells originating in the bone marrow (T 200 or HLe-1 in the human). Langerhans cells and indeterminate cells also express Fc-IgG and C_3 receptor sites,[18] and in normal epidermis, with the exception of acrosyringeal keratinocytes, are the only cells which express Ia (immune response-associated) or class II major histocompatibility complex (MHC) antigens (HLA-DR in man).[2,11,19,20] By contrast, at least in the mouse, Langerhans cells have been reported not to express class I MHC antigens. Human Langerhans cells express surface CD1a (T6),[21] and to a lesser extent CD4, antigens, which they share with cortical thymocytes and helper T lymphocytes, respectively. On the basis of double labelling studies, some authors have postulated the existence of different subsets of Langerhans cells/indeterminate cells of phenotype HLA-DR+T6+, HLA-DR+T6−, and HLA-DR−T6+; we were not able to demonstrate the presence of HLA-DR+T6−, and HLA-DR−T6+ subsets on flow cytometric analysis of double stained human epidermal cell suspensions pre-enriched for Langerhans cells.[14]

IMMUNOLOGICAL FUNCTION OF LANGERHANS CELLS

During the course of ultrastructural investigations of the cellular events occurring during contact hypersensitivity reactions in man and guinea pigs, Silberberg *et al.* noted close apposition between Langerhans cells and lymphocytes, as well as antigen-bearing Langerhans cells in dermal lymphatics and regional lymph nodes.[22] On the basis of these observations, they proposed that Langerhans cells might play an important role as antigen-presenting cells in cutaneous delayed type hypersensitivity reactions. Discovery that Langerhans cells bear Fc-IgG and C_3 receptors[18] provided additional evidence suggesting that Langerhans cells were of monocyte-macrophage lineage. The classical experiments of Stingl *et al.*[11,19] established that rodent Langerhans cells do indeed function as specialized epidermal antigen-presenting cells, since they induce allogeneic and antigen-specific T cell activation in the epidermal lymphocyte reaction, and are required for epidermal cell-induced generation of cytotoxic T lymphocytes.[23] The antigen presenting capacity of Langerhans cells has subsequently been confirmed in humans.[24,25]

ROLE OF LANGERHANS CELLS IN DISEASE

The morphological findings of Silberberg *et al.*,[22] confirmed and extended by numerous other authors (reviewed in Breathnach, 1986[26]), as well as the data on the antigen-presenting function of Langerhans cells,[11,19,23-25] have led to the concept that Langerhans cells play a critical role in the pathogenesis of allergic contact dermatitis, in that they pick up antigen in the skin and present it to lymphocytes in both the skin and draining lymph nodes. Other morphological studies (reviewed in Breathnach and Katz, 1985[27]) have implicated Langerhans cells in the aetiology of a variety of lymphocyte-mediated skin diseases in addition to allergic contact dermatitis. Langerhans cells have been regarded as interacting with helper T lymphocytes, or as specific targets for destruction by cytotoxic T cells, in lichen planus, cutaneous T cell lymphoma, allograft rejection and cutaneous graft-versus-host disease. The recent finding that IgE is present on epidermal Langerhans cells of patients with atopic dermatitis, but not those of normal individuals, may partly explain the high frequency of positive patch tests to inhalant allergens in atopic dermatitis, and provide a potential bridge between immediate and delayed type hypersensitivity reactions in these subjects.[28]

Langerhans cells from patients with AIDS or AIDS-related complex are decreased in number and may demonstrate cytopathic changes associated with the presence of intracellular viral particles characteristic of HIV-I on electron microscopy; the Langerhans cells react with a monoclonal antibody specific for HIV-I core protein p17.[6] These findings suggest that Langerhans cells may harbour HIV-I. It has been proposed that certain domains of the CD4 molecule, present on the cell surface of both helper T lymphocytes and Langerhans cells, may act as specific binding or receptor structures for the retrovirus. Since Langerhans cells are thought to subserve a critical role in cutaneous immunosurveillance by virtue of their antigen-presenting capacity, infection of Langerhans cells by HIV-I may contribute in part to the immunodeficiency and infectious and neoplastic complications of AIDS.

EFFECTS OF PHYSICOCHEMICAL AGENTS

Glucocorticosteroids

The inhibitory action of topical corticosteroids on both the induction and elicitation phases of contact dermatitis reactions is thought to be, at least in part, the result of effects on the skin immune system. Studies in rodents and humans have shown that topical corticosteroids cause a dose-related reversible decrease in Langerhans cell surface markers (including membrane ATPase, Fc-IgG and C_3 receptors, Ia/HLA-DR antigen, T6 antigen and S-100 protein), and when applied to the ears of mice result in decreased Langerhans cell antigen-presenting capacity (reviewed in Ashworth *et al.*, 1988[29]). We have recently shown that topical steroids, applied twice daily to human forearm skin for 7 days, significantly decreased not only the number of HLA-DR/T6 (CD1a) positive Langerhans cells, but also the Langerhans cell-dependent capacity of epidermal cells to present antigen to lymphocytes; the effects of the various steroids on both of these parameters correlated with their potency as determined in the standard occlusive vasoconstrictor assay.[29]

Gamma radiation

Localized gamma radiation causes a dose-related reversible decrease in the number of Ia+, ATPase positive, or F4/80 antibody positive Langerhans cells in rodents (reviewed in Breathnach & Katz, 1985[30]). We reported that lethal whole body X-irradiation of mice results in a dose- and time-dependent decrease in the number, and alloantigen and antigen presenting capacity of, Ia+ Langerhans cells.[30] Thus X-irradiation of the skin may affect inflammatory and neoplastic processes not only by its anti-mitotic activity, but also by a direct action on the skin immune system.

Ultraviolet radiation

Exposure to ultraviolet radiation, both UVB and PUVA, during the induction phase of contact hypersensitivity reactions results in the development of specific unresponsiveness to the contact allergen in rodents and humans, which is mediated by antigen-specific suppressor T cells.[4,27,31] Although the inhibitory action of UVB and PUVA on the induction of contact sensitization is complex, it has been linked in part with the effects of these agents on Langerhans cells. UVB and PUVA cause a decrease in the number of Langerhans cells expressing membrane ATPase and Ia/HLA-DR antigens, associated with a decrease in epidermal antigen-presenting capacity secondary to impaired Langerhans cell function.[4,27,32-35] Ultraviolet irradiation of rodents has also been shown to lead to inability to reject transplanted tumours which are normally highly antigenic and readily rejected. Thus, abnormalities of Langerhans cell function, with a consequent failure of cutaneous immunosurveillance, may be involved in the pathogenesis of ultraviolet radiation-induced cutaneous malignancy.

CONCLUSION

Our concept of the importance of the Langerhans cell has changed radically during the last 100 years, and particularly during the last quarter of a century. Confines of space have precluded reference to the work of many authors who have made significant contributions to this field, but advances have, in many senses, been the result of a concerted international effort. The status of the Langerhans cell as the resident antigen presenting cell within the epidermis is now firmly established. A considerable body of evidence supports the view that altered Langerhans cell-lymphocyte interactions may be central to the pathogenesis of certain lymphocyte-mediated skin diseases, including allergic contact dermatitis, lichen planus, and mycosis fungoides. Therapies which are widely used in dermatology, including UVB, PUVA and topical steroid treatment, may also exert their effect in part as a result of their effects on Langerhans cells. It is still a matter of controversy whether these therapies destroy Langerhans cells or merely modulate expression of their surface markers. The fact that depletion of HLA-DR+ Langerhans cells following steroid therapy or ultraviolet irradiation is reversed with time, even when human skin is transplanted onto athymic (nude) mice, a system devoid of circulating precursors for human Langerhans cells, suggests that these agents induce modulation of Langerhans cell markers rather than bringing about cell death.[36]

Whilst many of the enigmas surrounding the Langerhans cell have been clarified, certain other questions relating to the biology of the cell remain to be answered. The suggestion that Langerhans cells may in some way control the growth and differentiation of a pool of neighbouring keratinocytes in an 'epidermal proliferative unit' has not been entirely refuted. Perhaps the most intriguing problem is that of the function of the characteristic intracytoplasmic granule. Although it is feasible to obtain highly enriched populations of Langerhans cells using 'panning' techniques or flow cytometric sorting, it has so far not been possible to establish long term *in vitro* cultures of Langerhans cells in order to obtain sufficient cellular material for the isolation and biochemical characterization of the granules; this must be a project for the future.

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Centenary Review

Diet and dermatology in 1888: the influence of H.Radcliffe Crocker

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We are what we eat, and the relationship between diet and disease has fascinated physicians for centuries. Some skin diseases, such as pellagra and scurvy, are due to specific dietary deficiencies, others such as the yellow palms of carotenaemia can be caused by dietary excess, and a variety of allergic, toxic and idiosyncratic reactions, including such oddities as Kwok's queaze (flushing after sodium monoglutamate) can also be traced to dietary factors. A recent revival of interest in the metabolic effects of essential fatty acids and trace metals such as zinc and lithium has stimulated research into the effects of dietary supplements in the treatment of dermatoses as diverse as atopic eczema, psoriasis, acne, systemic sclerosis, and leg ulcers. Some of this work will be discussed elsewhere,[1] but for this Centenary issue of the Journal I should like to review the subject of diet and the skin as seen through the eyes of a British dermatologist of 100 years ago, and incidentally to pay tribute to one of the great medical textbooks of the nineteenth century.

Radcliffe Crocker's textbook

Henry Radcliffe Crocker (1845–1909), chief dermatologist at University College Hospital, London, published the first edition of his magnificent work, *Diseases of the Skin*, exactly 100 years ago.[2] The book established his reputation as one of the leading dermatologists of his day, and it is clear from his long obituary notice in the *British Medical Journal*[3] that he was regarded as a pillar of the profession . . . 'if ever a man raised himself to the first place in his branch of medicine by industry and ability it was Radcliffe Crocker'.

He was certainly an astute clinician. Sir Archibald Gray gave him credit for the first descriptions of granuloma annulare, acne agminata, dermatitis repens, recurrent winter and summer eruptions and erythema elevatum diutinum[4] and he was also the first to note the presence of micrococci in the vesicles of impetigo.[5] In 1933, Arthur Whitfield wrote that 'his great volume remains, in my opinion, the best work on dermatology that has been produced in any language',[6] and more recently David Williams called it 'the Old Testament of our discipline . . . written in lucid classical English'.[7]

Crocker's elegant style and clinical acumen are nicely illustrated by his account of *Feigned Eruptions* (p. 242); 'Besides their legitimate use, various irritants may be fraudulently employed, chiefly by hysterical women, mendicants, soldiers, prisoners, or domestic servants, either with a sordid or mobid object of obtaining sympathy, or to avoid some irksome duties . . . the imposters are often successful in their object when there is an apparent absence of adequate motive. The following points will aid in detection; but let not the young physician expect credit for so doing, as the friends of the hysterical one are often almost as angry with the discoverer as they are with the perpetrator of the deceit'. There follows an excellent description of the clinical features of dermatitis artefacta, with several case-reports, including details of the agents used (turpentine, black-lead, mustard, cantharides, nitric acid etc.).

Crocker's experience and erudition are apparent on almost every page of his book, and though some of his notions may seem quaint to the modern reader, we must bear in mind the tremendous diagnostic and therapeutic confusion which existed 100 years ago, before the days of frequent biopsies and double-blind controlled trials.

The pathogenic role of diet and the gastrointestinal tract

In the late nineteenth century, many Continental dermatologists believed, with Hebra, that most skin diseases were caused by external agents, whereas British dermatologists, including Hutchinson, tended to follow Bazin's 'diathetic' view that constitutional predisposition played a major pathogenetic role.[8]

Crocker's views on the vital importance of diet in dermatology followed from his conviction that most skin diseases are secondary to internal disease; (p. 1) 'In some instances the skin disease is the primary event, and the general disturbance secondary to it, as in cases of extensive and severe skin diseases, which lead to vital depression, febrile disturbance or marasmus. On the other hand—and this is by far the larger class—some internal derangement, functional or organic, as in disturbances of the alimentary canal, the uterus, and ovaries, the kidney etc., leads directly or indirectly to the skin disorder'.

Of all these 'internal derangements', the most important to the dermatologist was dietary disturbance; (p. 19) 'Food, improper in quality or quantity, is an important factor in the production of a large number of diseases. It may do this by lowering nutrition generally, or by its directly irritating effects on the gastrointestinal mucous membranes. Or it may be of a quality which promotes fermentation in the alimentary substances in the stomach. As examples may be given the effect of taking food containing branny particles, such as brown bread, oatmeal, etc. on eczematous and urticarial patients, and the influence of beer, pastry etc. in promoting fermentation. More direct is the gastric irritation produced by shell fish, especially mussels, which excite violent urticaria in some people'.

Crocker's attitude is illustrated by his ideas on eczema. He recognized of course that there were many external causes of eczema, but when it came to endogenous eczema, he had a gut feeling; (p. 106) 'the complex condition known as dyspepsia is very frequently present, and the bowels are very often disordered, either from constipation or from diarrhoea or deficient bile. This may, however, be simply a concomitant, an acute eczema being often associated with pale motions and furred tongue, and as the two often come on simultaneously it is reasonable to suppose that there is catarrh both of the alimentary canal and of the skin'.

It is possible that a few of Crocker's patients with so-called 'eczema' and malabsorption were actually cases of dermatitis herpetiformis with coeliac disease, but this cannot have accounted for many cases, since he was familiar with *hydroa pruriginosum*. Tilbury Fox Crocker's predecessor at U.C.H. had described dermatitis herpetiformis under this name in 1880,[4] and Crocker also refers to Duhring's classic paper on the subject, published in 1884,[9] in his own account of hydroa.

The mechanisms by which the gastrointestinal tract produced inflammatory skin disorders were obscure, but (p. 106) 'For my own part, I think they are all instances of irritation of the alimentary canal, which reflexly irritates the nerve centres, and produces dilatation of the capillaries of the region affected'. For Crocker these obscure 'reflexes' secondary to 'gastrointestinal derangement' evidently provided a similar straw to that which modern authors clutch when they write of obscure 'T cell imbalances' secondary to 'immunological derangement'.

Crocker was aware of course that other internal organs could affect the skin. Liver disease was in his experience associated with xanthoma, jaundice, urticaria and severe pruritus, asthma was associated with urticaria, eczema and ichthyosis, and diabetes mellitus also caused many problems; (p. 25) 'Kaposi found xerosis, pruritus, urticaria, acne cacheticorum, roseola and erythema, eczema especially of the genitalia, balanitis and vulvitis, boils and carbuncles, gangrene and to these must be added the rare xanthoma diabeticorum'. He was less impressed, however, by the role of the kidney, and he was critical of M.Augagneur who had described 44 cases of albuminuria associated with miscellaneous skin diseases; (p. 24) '... few would assign any aetiological connection with at least the first three dozen'.

Time and again throughout the book however, Crocker returns to his theme that dietary disturbance is the major cause of inflammatory skin disease: 'The digestion should always claim our first attention. The diseases most directly connected with disturbance of the alimentary canal are urticaria, acne rosacea, and eczema, pruritus both general and local, e.g. pruritus ani, but all inflammatory diseases are likely to be aggravated by it.'

Even differences due to age and sex were susceptible to a 'gastrointestinal' explanation; (p. 21) 'The different habits of the two sexes also play a part. Thus, the minor form of acne rosacea is more common in women, from their greater liability to dyspepsia and constipation, owing to their sedentary habits; on the other hand the worst forms are seen in men, from their more frequent intemperance'. (p. 21) 'In infancy, from the ease with which the alimentary canal is deranged there is a greater liability to eczema or urticaria.'

In short, dietary defects could account, at least in part, for almost every inflammatory dermatosis, and for quite a few of the others, such as lentigo, which Crocker recognized in a 'symptomatic' form secondary to atrophoderma, old age or xeroderma pigmentosum; (p. 340) 'Defective nutrition is the chief cause of symptomatic lentigo'.

The therapeutic role of the diet

Given his views on the pathogenesis of skin disease, Crocker's advice on the use of diet in treatment is fairly predictable; (p. 33) 'Dietary (sic) plays a most important part. It must always be borne in mind that most inflammatory affections have an intimate sympathy with the gastric mucous membrane, and whatever irritates that, aggravates the skin trouble'. He goes on to give a long list of dietary prohibitions '... all highly spiced food ... condiments of all kinds ... salted foods are often also injurious ... oatmeal and bran-containing preparations do not suit those who have acute inflammatory affections ... infants and young children with gastrointestinal catarrh can seldom digest starchy food, which should therefore be avoided or given sparingly, and then with maltine.'

Detailed and specific dietary instructions were given for many diseases. For rosacea, for example; (p. 589) 'Fermentable articles of diet should be avoided; such as sweets, pastry, rich gravies, thick soups etc.... .those kinds of cocoa in which the superfluous fat is removed are preferable to the cruder or starchy kinds'.

Even when the disease had a specific treatment, attention still had to be paid to the diet. Thus for lice; (p. 705) 'In marasmic subjects, suitable measures in the way of feeding, cod-liver oil, and the removal, if possible of the cause of emaciation, are valuable adjuncts.'

We must remember though that the nutritional state of the majority of the urban population in Victorian England was very different from that of the modern Welfare State. Those were the days of soup-kitchens and industrial grime, poverty and pea-soup fogs, when sunshine was short and gin was mother's ruin.

Alcohol. This of course provided solace for many otherwise intractable diseases, but Crocker's views on the subject are clear; (p. 33) 'The question of alcohol is one on which most patients are very anxious. Speaking broadly, as a rule, the less the better; alcohol dilates the vessels of the skin and is, therefore, contraindicated in inflammatory affections, in which it generally aggravates the pruritus and increases the hyperaemia'. He was too good a clinician though to have any absolute prohibitions, and every case had to be considered on its merit; (p. 34) 'In elderly people it is seldom wise to break up too suddenly the habitual use of alcohol, or indeed almost any habit not positively deleterious'. Even for rosacea, alcohol was not absolutely contraindicated in every case; it could be taken (p. 589) 'in very small quantities in atonic dyspepsia at the beginning of a meal'.

If alcohol was essential though, careful attention was to be paid to the quality; (p. 34) 'a very small quantity of a pure spirit well diluted, or one of the lighter wines, such as claret or hock, which must however be perfectly sound and mature, while as a rule the stronger wines, such as port and sherry and the imperfectly fermented products such as beer, porter and the sparkling wines, are more or less injurious'.

He was not afraid to contradict previous oracles on this or any other subject; (p. 586) 'According to Hebra, rosacea is, in spirit drinkers, more frequently limited to the nose, and consists of vascular dilatation and seborrhoea; while in wine drinkers the redness is diffuse and seldom limited to one region, and the whole face is bloated; and in those who affect beer, cyanide thickening with small nodules and pustules is more frequent. These distinctions are probably fanciful'.

Mineral waters. The current fad for mineral waters on the better-quality dining tables would no doubt have met with Crocker's approval. In a six-page appendix to his book, he details the various types of waters (purgative, alkaline, ferruginous, arsenical, etc.) which were then available at the spas of Europe, from Aix-les-Chappelle to Strathpeffer. Although the springs were widely acclaimed for the treatment of many skin diseases, including psoriasis, syphilis, tuberculosis and eczema, Crocker appears sceptical about the curative powers of the water itself, noting dryly that 'some of the waters are adapted for weak digestions and long purses'.

In his view, the benefits of hydrotherapy were more holistic; (p. 716) 'Far more efficacious than swallowing the imported waters is a visit to the spas themselves. It must, however, be borne in mind that there are many other elements beside the composition of the waters which make for success in the restoration of the patient. Among these are the climatic conditions, and the consequent change of air and scene, the regimen and regular hours as well as the withdrawal from many of the temptations of society life'.

Previously, I had always imagined that it was these society temptations, rather than the foul-tasting water, which provided the major attraction of these otherwise spartan spa-towns, but I bow to Crocker's greater knowledge, for he was far from naive; (p.717) '. . . and last, but not least is the influence of hope and faith engendered in the carrying out of a new treatment in which there appears to be a little mystery, and in which the very expense and trouble stimulate the patient to do all that he can to get well, instead of carrying out the treatment in the half-hearted way in which patients at home, are too apt to subordinate the means of cure to their engagements and convenience'.

Specific dietary factors

Crocker also had interesting views on the role of specific dietary factors in some skin diseases.

Pellagra. In 1888 pellagra was regularly seen in parts of Spain, Italy and France; (p. 76) 'The disease occurs almost exclusively (90%) among the poorest peasants of the districts affected, but though it is predisposed to by poverty and bad hygiene generally, the immediate cause is the toxic influence analogous to ergotism, produced by eating decomposed or fermented maize, during which, as Lambroso's experiments show, a fatty oil (maize oil) and an extractive 'pellagrozein' are produced, and the administration of these to men and animals excited pellagrous symptoms in them'.

Crocker realized that patients with pellagra, improved following 'removal into good surroundings and good feeding', but in his view 'of all remedies, arsenic is the most effectual'.

Leprosy. At that time, leprosy was endemic in Norway, and in parts of southern France and Spain, but its cause was disputed, even though Hansen had identified the lepra bacillus in 1873; (p. 470) 'Eating fish, especially if putrid, is supposed by some high authorities to be the cause of leprosy, but since in many countries where, either from religious prejudice or other circumstances, no fish is eaten, yet leprosy is rife, this theory must be regarded as untenable'. One of the 'high authorities' who still clung tenaciously to the 'rotten fish' theory was the great Jonathan Hutchinson of the London Hospital.

Sweat. This was another fishy subject in the late nineteenth century; (p. 560) 'Phosphorescent sweat is a curious rarity. It has been observed after eating phosphorescent fish, while Koster records a case where the body linen became luminous after violent exertion'. (p. 559) 'Green sweat due to copper which has been taken into the system by the food, drink, or air, in particles or fumes, is seen mainly in copper workers'.

Acne vulgaris. Crocker did not promote the idea that greasy foods cause acne vulgaris, but he accepted the role of constipation, dyspepsia and (p. 581) 'all debilitating causes, too rapid growth and perhaps masturbation'. Scurvy, in his experience, not only caused acne, but modified its course, 'leading to freer suppuration than usual'.

For the treatment of seborrhoea; (p. 570) 'Iron and cod-liver oil are the two remedies of most frequent utility, but the alimentary canal often requires preliminary attention'.

Lichen scrofulosus. This was defined as (p. 222) 'very small chronic inflammatory papules of a red colour, fading to that of the normal skin, disposed in groups or circles, and occurring mainly in scrofulous subjects'.

The treatment 'is simple and effective. Cod-liver oil, internally and externally, always removes the eruption. Externally it must be not only rubbed in, but the skin kept constantly soaked in it. This is Hebra's treatment, and answers well, but is necessarily disagreeable for all parties concerned.'

In common with other dermatologists of his day, Crocker also recommended cod-liver oil for lupus vulgaris and scrofuloderma.

Herpes progenitalis (genital herpes). The cause was unknown, but Crocker noticed that it tended to be associated with other venereal diseases. This was one condition which he did not ascribe to diet, though apparently others did; (p. 149) 'The recurrences are generally determined by local irritation, especially coitus, passing a catheter etc. For my own part, I am more inclined to ascribe it to such local causes than to internal disturbances, as French authors assert, such as arise from the gouty diathesis, excesses in eating or drinking, dyspepsia, or exhaustion from any cause'.

Pustula maligna (anthrax). The anthrax bacillus had been identified by 1888; (p. 267) '. . : flies are sometimes the medium of its conveyance, while the flesh, if imperfectly cooked, and milk or butter from the diseased animals, have produced it in rare instances'.

Furunculus. Crocker advocated several treatments for furuncles, but his favourite seems to be (p. 233) 'fresh yeast, half a wineglassful to be taken night and morning. This is a popular and good remedy, though its *modus operandi* is not clear, unless we suppose that the yeast organism has the power necessary for appropriating some pabulum necessary for the existence of the furuncle organism. Stout or port wine is sometimes a useful adjunct in the debilitated, though it should not be given indiscriminately'.

Filariasis. Modern dermatologists who think of their specialty as unheroic might like to consider Crocker's treatment of elephantiasis arabum; (p. 336) 'The scrotal tumours may be removed by the knife; even those over one hundred pounds have been successfully removed. . . In the leg an attempt has been made to starve the growth by ligaturing the femoral artery, but has seldom been permanently successful'. Even in these cases however, Crocker does not neglect the diet, for the treatment had to be 'combined with an exclusively milk diet'.

Crocker's influence on British views on diet and dermatology

In 1888 there were many opinions on the role of diet in the aetiology and treatment of inflammatory skin disorders, and Crocker's views were neither unique nor entirely original. Tilbury Fox, for example, had suggested that eczematous eruptions were analogous to the catarrhal inflammation of the mucous membranes, and others had noticed that subjects with the 'eczematous diathesis' tended to suffer from 'catarrh' of the respiratory and digestive tracts. Few of Crocker's contemporaries, however, embraced the 'gastrointestinal irritation' theory with quite his evangelical fervour.

Kaposi, for example, in his rival textbook,[10] accepts that dyspepsia and excessive alcohol consumption predispose to rosacea, but he briskly dismisses the role of gastrointestinal irritants in eczema; (p. 362) 'We are never opposed to the ingestion of salted or spiced food, cheese, caviar etc. because they increase neither the eczema nor the itching'.

Anderson, in his book,[11] conceded that (p. 135) 'a too liberal diet, and too simulating food and drink, by deranging the digestive organs, may predispose to eczema, though certainly not so readily as a diet deficient in nutritive properties'. He quotes the case of a 'dreadfully emaciated' 15-month-old child with 'eczema impetiginodes' (infected eczema); (p. 154) 'The case looked hopeless and had been given up by the previous attendant'. The response to 5 weeks of Anderson's internal treatment was dramatic. 'Here then is an instance of an infant cured of a frightful eczematous eruption and rescued from the jaws of death, by the internal administration of cod-liver oil and iron alone'. One doubts whether topical steroids would have done so well. The eczema which Anderson saw in Glasgow was a disease of 'the chlorotic (anaemic), the rachitic, the scrofulous and the debilitated'.

Jamieson of Edinburgh, as we might expect, took a different view.[12] He postulated that eczema was caused by dietary changes consequent upon civilization, such as the increased refining of flour and the artificial feeding of beef cattle. Flour and beef were not the only dangers which Jamieson's patients had to fear however; (p. 227) 'Most harmful of all, in the sense of predisposing to eczema, is the abuse of tea. This, by retarding tissue change, and lessening waste leads to the accumulation of effete products in the system'. The results of such a dangerous life-style were likely to be serious . . . 'the imperfect removal of excrementitious matters already existent will be further complicated by the presence of the products of incomplete combustion'. Note Jamieson's appropriately flatulent style, which contrasts with Crocker's crispness.

A few years later, Walker of Edinburgh[13] was to put the wind up even more Celtic kilts with this stern warning; (p. 118) 'Porridge is regarded by many as harmful. Insufficiently cooked, as it too often is out of Scotland, it is undoubtedly as bad for patients with eczema as it is unpalatable to all'.

Eventually, however, it was Crocker's views which prevailed, and his advice on the avoidance of gastrointestinal irritants was regurgitated almost verbatim in many British textbooks until comparatively recent years. The textbook I used as a student,[14] for example, had this to say on the treatment of eczema; (p. 188) 'The following foods should, in general, be forbidden. Pork, bacon, ham, sausage, cheese, rich gravies, highly spiced foods, salted and tinned meats, pastries, tomatoes and sharp acid fruits. The use of sugar should be restricted, and a salt-free diet has a beneficial effect in many eczemas. Alcohol should be rigidly forbidden, strong coffee and tea should be limited'.

Few dermatologists would now swallow Crocker's dietary disquisition, even with a pinch of salt, but his *Diseases of the Skin*, which ran to three editions, still provides a refulgent landmark in the field of British dermatology texts. It is salutary to reflect that virtually all our predecessors who have produced textbooks of lasting value have made serious conceptual errors, and it is unlikely that our present textbooks will not cause some amusement in 100 years time.

It is worth pointing out too, that Crocker's general advice on diet, which might be summarized as—'Alcohol, the less the better; take a bland nourishing diet, but nothing which might cause gastrointestinal irritation' is unlikely to be seriously criticized today, whether or not it has any influence on the course of inflammatory skin disease. His lasting influence on British dermatologists may be judged by the fact that even in the 1970s, some Harley Street men were known to issue detailed instructions on food and drink to their patients which were almost identical to those issued by Crocker, and for all I know, they may do so still. After all, solicitude and careful attention to the detailed requirements of the individual patient are not without therapeutic effect.

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Centenary Review

The effect of light on the skin

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I would like, as a dermatologist with an interest in cutaneous photobiology, to try to assess what has been achieved in the study of the effects of light on the skin and how this knowledge has assisted the present day investigation and treatment of patients with suspected photosensitivity.

I came into dermatology in the 1950s at the time when the specialty was already leaving the morphological era and enquiring into the reasons why the skin might react in certain ways, including in response to exposure to light. About that time Magnus, Porter and Wright[1] were developing the irradiation monochromator which was to be the first step in putting the new discipline of cutaneous photobiology on a firm basis. The ability to confirm photosensitivity, suspected on clinical grounds, by the demonstration on phototesting of abnormal responses to specific wavelengths of the sunlight spectrum was to make the next 30 years a time of progress in comparison with the first part of the century. Although there were many good clinicians, it had not been possible to separate out the various photodermatoses, a necessary step before any study of the causation and mechanisms involved could be attempted. We now recognize the photodermatoses as clearly defined entities, but this was not always so and for a long time there was confusion, in part due to the use of the group term 'polymorphous light eruptions' which later were shown to contain a number of different forms of photosensitivity reactions. In particular, this delayed the recognition of the group of photosensitivity reactions which are now known to be caused by photoactive substances which reach the skin either from external contact, leading to a photocontact reaction, or via the circulation following ingestion, causing the drug induced photosensitivity eruptions.

Thus, it was necessary to develop irradiation sources which provided selected wavelengths of emission of predictable purity, and in measured amounts, which could be used to confirm a diagnosis of cutaneous photosensitivity suspected on clinical grounds. Although the ideal source would have been that of natural sunlight, this was soon found to be unpredictable and, therefore, unreliable. Thus artificial irradiation sources such as the mercury and carbon arc were constructed and used in phototesting, particularly prior to the mid 1950s. However, these sources also had their limitations in that they did not provide a continuous emmision of the sort of wavelength mix to be found in natural sunlight. It was, therefore, not until the production of the xenon arc[2] as the irradiation source, which was to provide for the first time reliable solar simulation, that our present day technique of phototesting arrived. Its use in combination with a monochromator was applied by Magnus to the first detailed study of the photodermatoses.[3] Thus it became possible not only to confirm a clinical diagnosis of photosensitivity, but also to determine the responsible wavelengths, i.e. the action spectrum of the eruption, important in the differential diagnosis of the various photodermatoses and later in the search for causal factors and in the study of the mechanisms involved. The technique of wavelength selection was later facilitated by the introduction of the grating monochromator[4] and over the years other photobiology centres have produced their own modifications and refinements.[5–8] In particular, mention should be made of the 'solar simulator' developed and used in Philadelphia by Berger and Urbach[9] which provided a broad-band irradiation source similar to natural sunlight, which was to prove particularly useful as a screening method for establishing the presence of photosensitivity and later in the technique of photopatch testing.

The technique of phototesting was, however, found to have its own limitations that were separate from those of the irradiation sources. Usually, the end-point used was that of minimal erythema, either immediate or delayed, and not necessarily the reproduction of the clinical eruption with its range of morphological changes. This has led to the requirement in certain photodermatoses for variations in the technique, such as the use of repeated irradiation, the selection of different skin sites, variation in the size of the area irradiated, and so on. This limitation has not prevented the technique continuing to be widely and successfully used, although eventually it is going to become necessary, despite the inherent difficulties, to try to assess the response of the clinically affected exposed skin as well as the unaffected covered back skin. This will not be easy.

How far, therefore, have we come in our understanding of the idiopathic photodermatoses such as polymorphic light eruption, actinic prurigo, hydroa vacciniforme, and solar urticaria, and of the mechanisms involved in the photosensitivity eruptions peculiar to each? Probably few would now disagree that polymorphic light eruption and actinic prurigo can be differentiated on the basis of features such as those of morphology, distribution, and seasonal incidence. Hydroa vacciniforme is also a separate entity, particularly evident since the discovery by Magnus and his colleagues of erythropoietic protoporphyria in the 1960s,[10] and this despite the fact that some otherwise classical examples of actinic prurigo may show small vesicles and superficial scarring, presumably the hydroa aestivale of earlier texts. Despite some variation in its presentation, as it affects those in Latin America, North America and in Canada, actinic prurigo seems to be a clinical entity, although whether in time it is found that there are photosensitivity mechanisms common to the other idiopathic photodermatoses remains to be seen. We also know by phototesting that the responsible wavelengths involved are a broad waveband and that the long wavelength UVA may be particularly relevant in all three conditions, important not only for the correct selection of topical photoprotective agents, but also for future study of the mechanisms involved. There must be few dermatoses, be they light-induced or otherwise, which affect so many of the population in different parts of the world as does polymorphic light eruption, suggesting that we may have more than one condition which will some day tell us as much about normal as abnormal responses to sunlight; the suspicion that immunological mechanisms are involved in polymorphic light eruption and in actinic prurigo has yet to be substantiated. Solar urticaria is also an entity distinct from those photosensitivity responses which also may have a wealing component, such as polymorphic light eruption, porphyria, and tar photocontact reactions. On the basis of an action spectrum, which is usually broad and commonly involves UVA and visible wavelengths, immunological mechanisms are said to be more likely to be involved when the response is confined to the shorter UVB or to the visible wavebands. What, however, remains to be determined is the structure in the skin which absorbs the sunlight leading to the photosensitivity reaction and the release of the various chemical mediators following mast cell damage. There is, however, in solar urticaria, no known chromophore nor is there a definite photoactive substance produced in the skin by the irradiation. Until recently, it has been a distressing condition and relatively unresponsive to treatment, but it is now apparent that PUVA is effective and that there may well also be advantages in combining the phototherapy with non-sedative type I receptor blocker antihistamines.

However, there has been more progress in the study of photoactive substances, particularly in the photocontact reaction, but also following ingestion, as in drug induced photosensitivity eruptions. A study of these substances has not only improved our understanding of the mechanisms of phototoxicity and to a lesser extent photoallergy, but has helped in the development of photochemotherapy. Early in the century, photocontact reactions were recorded to substances such as fluoresceine dyes,[11] coal tar,[12] eau de cologne,[13] and to plant materials,[14] but those investigators did not have the range of laboratory techniques later to become available[15] which allowed for the assessment of the phototoxic potential of such substances, and which also indicated the possible site of the photosensitivity reaction, be it for example either in the cellular DNA or membrane; neither had the photopatch test been developed where the same potential could be assessed by testing of human skin. However, in some instances they recorded persistent pigmentation following such photocontact reactions as a sequel to an initial erythema response, and such pigmentation was later to be appreciated as important in the differential diagnosis of melanoderma and in facial melanosis in particular.

The photocontact reaction was also later shown to play an important part in the development of the chronic reactive state of the skin found in photosensitivity dermatitis (photosensitive eczema, actinic dermatitis) and actinic reticuloid syndrome[16] where the skin is exposed to repeated stimuli which probably make use of both immunological (contact allergy and photoallergy) and non-immunological (phototoxic) mechanisms. Over the years, firstly the sulphonamides, then the phenothiazines, the salicylanilides, and more recently the Compositae family of plants and allied fragrance materials, were recognized as being similarly involved. It was, however, with the halogenated salicylanilides photocontact epidemic of the early 1960s[17] that an ability to investigate such cases became an urgent requirement. It might be said that it was this epidemic, above all, which gave the impetus for the subsequent development of clinical photobiology. Most of those affected by such photocontact reactions got better relatively soon after avoidance of exposure; a minority, however, went on to develop chronic photosensitivity dermatitis for reasons that are still not clear, although I believe there is more to be said in favour of the multiple insult theory of both contact and photocontact nature, than the alternative suggestion that it is simply a sequel of 'photoallergy'. It remains to be determined, however, how the contact allergic reaction affects the photosensitivity, be it phototoxic or 'photoallergic', and what leads to the chronic atypical cellular response which, along with a pseudo-lymphoma morphology, allows the use of the term actinic reticuloid.[18] This syndrome has probably always been around, with peaks of activity dependent on variations in exposure to environmental photosensitizers and allergens. It is likely to continue to be so and will thus be available for the study of the inter-relationship in the skin between these various factors and perhaps even the border-line between chronic inflammatory responses and the reticuloses. Thus, this form of chronic photosensitivity has much still to tell us.

Most of the attention has been directed towards the problem of externally induced photosensitivity, and it is only in relatively recent times that the importance of similar photosensitivity, induced this time by a drug which has reached the skin via the circulation following ingestion, has received attention. However, in the early years, dermatologists did also recognize that ingested photoactive substances could photosensitize, such as eosin used in the treatment of epilepsy,[19] and a dye Rose Bengal which was used in the assessment of liver function and normally rapidly excreted, could on occasions be retained by some subjects long enough to reach the skin in sufficient amounts to induce photosensitivity, as was to be found later in the 1930s with sulphanilamide. What the salicylanilides did for the 1960s and 70s benoxaprofen[20] has probably done for the 1980s in drawing attention to the fact that a number of substances used in medical therapeutics such as sulphonamides, tetracyclines, phenothiazines, thiazide diuretics, quinine, and the non-steroidal anti-inflammatory group, are potentially photoactive and as such can cause cutaneous photosensitivity in both acute and chronic forms. For all the most commonly involved drugs the clinical aspects and responsible wavebands (action spectrum) have been determined and the evidence so far would favour the involvement of a phototoxic mechanism. Also of interest is the similarity between the clinical features of erythropoietic protoporphyria[10] as it presents in Britain, and the phototoxic reaction induced by the drug benoxaprofen[20] with its symptoms and signs imbalance, where the severity of the symptoms greatly outweighs the resultant changes in the skin and where, in both, the photosensitivity probably maximally affects the cell membranes. What has yet to be clarified, however, is why only a small number of those taking such drugs and in the same amounts do develop cutaneous photosensitivity. It seems probable that the extra amounts required to induce photosensitivity, and which accumulate in the skin, are a feature of the way that the individual subject deals with the drug, and of its pharmacokinetics, based on the assumption that most people would develop this phototoxic reaction if there were sufficient amounts of the suspect photoactive substance present in the skin along with exposure to sufficient light of the appropriate wavelengths. However, what in my experience does not seem to happen, is the development of chronic photosensitivity, i.e. persistent light reaction of some years duration continuing after the causal drug has been withdrawn, although this undoubtedly can occur for a period of months at the most with some drugs and in some subjects. In this way, ingested drug induced photosensitivity differs from that resulting from external exposure induced photosensitivity where a persistent light reaction is said to occur for a period of years after the exposure has been avoided, although in this instance it is often difficult to be certain that such contact and photocontact substances have been avoided.

Sunlight has long been used for its curative properties, for example by Finsen at the end of the last century in skin tuberculosis, and even as photochemotherapy by the Ancient Egyptians for the treatment of vitiligo following ingestion of the seeds of the plant *Ammi majus* which, unknown to them, contained a photoactive psoralen, later to lead to the psoralen photochemotherapy (PUVA) of the present day.

UVR has also been used for many years in the treatment of common disorders such as acne and psoriasis, often, however, without much attention being paid to the emission spectra of the UV light sources used. Where we have progressed, therefore, in recent times is in the more rational selection of the wavebands used, by relating this to the action spectrum of the therapeutic effect. An example of this is the current use of a waveband which peaks around 311 nm[21,22] in UV phototherapy of psoriasis, which also has the added advantage of minimizing the sunburning and carcinogenic action of the more conventionally used shorter wavelengths. It is probably only a matter of time before the specific action spectrum for the effect of PUVA in psoriasis will be determined, and the application of this information should allow the more rational and safe use of the total life irradiation dose. It is surprising also, that, some years after the first record of the photodegradation of bilirubin, and the application of this to the use of blue light phototherapy in the treatment of neonatal bilirubinaemia,[23] we should not have seen a similar application to other chemicals; but it may be that we are now about to do so as evidenced by the recent demonstration of the photodegradation in the skin of the antibiotic ciprofloxacin,[24] an unwanted and previously unsuspected effect of light. In much the same way, it has been known for a long time that haematoporphyrin will collect in tissue, particularly if it contains rapidly growing cells, as in a malignant tumour, and that such growth could be suppressed following irradiation with the appropriate waveband of light, and yet its application has been delayed until the development in recent times of lasers in combination with fibre optics[25] which facilitate the internal presentation of the irradiation. Such photodynamic therapy will probably now play a progressively increasing therapeutic role in the years to come.

Ultraviolet, whether from natural sunlight or from artificial sources, is a carcinogen, both on account of its effect on cellular DNA and probably also by means of immune suppression. We now know, at least in the experimental animal, that exposure of the skin to UVR will produce not only local, but also systemic immune suppression.[26] Also, xeroderma pigmentosum, which has an enzyme deficiency of cellular DNA repair after UV exposure, leading in time to widespread skin malignancies, also manifests impairment of immunity in that the development of contact hypersensitivity in sun exposed skin is markedly impaired compared with normal control subjects, and that the degree of this immunological impairment is directly related to the severity of the disease.[27] Since Cleaver in 1968[28] first described the excision repair defect in xeroderma pigmentosum, this condition has become a model for the study of photocarcinogenesis, with the demonstration of the variable features of its various complementation types, including milder forms which may present in the school aged child only as persistent sunburn erythema and localized freckling, as well as the later onset pigmented xerodermoid[29] where the defect is at the post-replication repair stage. Also, the demonstration of the repair defect in cells obtained by amniocentesis in the prenatal period can now provide important information to be taken into consideration in deciding on the justification or otherwise for termination of pregnancy.

Despite the problem of photocarcinogenesis, the sun continues to be worshipped, and in moderation this can only be a good thing. However, the greater ease of foreign travel, whereby the Caucasian and particularly the Celtic skin is exposed to sunlight of very different quantity and quality against which its protective mechanisms are inadequate, along with the current somewhat uncontrolled use of sunbeds and solaria, remains a cause for concern.

What then has been achieved? A diagnosis of photosensitivity can now be made with confidence and the relevant photodermatosis identified, based on clinical features supported by the results of investigative techniques, some of which are more reliable than others. Although we still have a group of idiopathic photodermatoses, much has been learned of the importance of photoactive substances, irrespective of whether they reach the skin from external contact, following ingestion, or as a metabolic product. We still do not know what mechanisms are involved in the light-induced chronic poikiloderma of some of the genodermatoses or why, for example in erythema multiforme or in chicken pox, lesions may only appear on skin which has been exposed to sunlight, nor what are the mechanisms involved in the photosensitivity reactions of the idiopathic photodermatoses, such as polymorphic light eruption, actinic prurigo and hydroa vacciniforme, and whether these mechanisms are all different or whether there is a common feature. Perhaps, however, we should not be too despondent when one thinks back to the confusion that reigned as recently as 30 and some less charitable might even say 20 years ago. Also, we know the shortcomings of the use of topical photoprotective agents, which have been prescribed without their selection being based on the knowledge of the action spectrum of the eruption and the protective capacity of the absorption spectrum of the preparation. We know from controlled trials the limitations of currently available systemic photoprotective agents such as the antimalarials and beta-carotene. Also, that it is no longer acceptable to randomly apply a topical sunscreen and proceed to investigations such as phototesting and patch testing, without first suppressing the reactive skin, which, in those most severely affected, will require temporary nursing behind roof-mounted blinds made of materials which cut off the majority of the relevant wavelengths. Above all, we have the bonus of phototherapy and photochemotherapy, regimens which are being improved all the time so as to maximize the therapeutic action and to minimize unwanted site effects, the benefits of which we must continue to provide based on a rational balance of advantages and risks. Also, there is an increasing understanding of the reasons for the development of cutaneous photosensitivity and particularly that resulting from environmental photoactive substances, be they the responsibility of the cosmetic or pharmaceutical industry or simply the fault of the chrysanthemum in the greenhouse or the lichen covered logs in the woodshed.

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Centenary Review

From mushrooms to molecular biology. 100 years of skin tumours with particular reference to cutaneous lymphoma

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'The clinical forms of the sarcomata of the skin are very manifold'. Thus wrote Dr Funk of Warsaw in his introduction to two articles on skin sarcomas which appeared in the first volume of the *British Journal of Dermatology*.[1] There are few of us today who would disagree with this sentiment. In his papers Dr Funk discussed Kaposi's sarcoma, leukaemia cutis and mycosis fungoides together with various other skin malignancies, and his account makes fascinating reading.

At about the time of this publication the partition of Africa was nearing completion, Bruckner and Mahler were at their most productive, John Dunlop had invented the pneumatic tyre and the Eiffel Tower was being constructed. James Frazer was preparing his work, *The Golden Bough*, in which he suggested that there was a natural progression from magical to scientific belief systems. Such a progression can also be followed in our concepts of cutaneous tumours during the last 100 years.

By 1888 various forms of skin malignancy had been clinically described with great expertise, treatment regimens still used today had been recommended and links between some tumours and exogenous carcinogens had been delineated. Kaposi had described his cases of idiopathic multiple pigmented sarcoma of the skin (1872), Pemberton had recommended wide local excision and lymph node dissection for malignant melanoma (1858) and Hutchinson in 1888 suggested an aetiological connection between arsenic and cutaneous keratoses. The story of the development of our understanding of tumours of the skin since this time is a revealing and fascinating one. Dramatic progress has been made in our concepts of melanocytic tumours, non-melanoma skin cancer and soft tissue sarcomas during the last 100 years and even a brief review covering all these fields would fill many issues of this Journal. I propose, therefore, in the rest of this article to discuss cutaneous lymphoma, as an illustration of how our clinical observations and laboratory investigations have developed during the last century.

Early concepts of cutaneous lymphoma were based on careful clinical and light microscopic morphological descriptions of patients' lesions together with observations of the clinical course of the disease process. The meticulous detail and clarity with which these descriptions were expressed are very impressive and one only wishes that clinical papers today were as carefully written.

Alibert, whose contributions to the literature on cutaneous lymphoma preceded those of Dr Funk by more than 80 years, first used the term mycosis fungoides in his description of the patient Lucas, who suffered from a chronic scaly skin disorder and who eventually developed small skin tumours resembling mushrooms.[2] The natural progression of the disease from an early non-specific eczematous or 'pre-mycotic' phase through plaque lesions to a tumour stage was suggested by Bazin[3] and in 1885 Vidal and Brocq described a form of mycosis fungoides presenting as tumours without any evidence of preceding skin disorder, coining the term 'tumeur d'emblée'.[4] Four years after the founding of the *British Journal of Dermatology*, an erythrodermic variant of mycosis fungoides was reported by Besnier and Hallopeau. This may be the first recorded description of what we would regard today as Sézary syndrome.[5] Despite these detailed clinical descriptions of mycosis fungoides there was no general agreement regarding the nature of the disease process until the beginning of the twentieth century. An infectious aetiology was supported by many eminent physicians of the time including Koebner and Auspitz, and workers suggesting that mycosis fungoides was a neoplastic process were in the minority. I am glad to report that Dr Funk of Warsaw was a member of this minority, stating 'Not a single symptom compels us to adopt the infectious nature of mycosis fungoides . . . we consider ourselves justified in looking on mycosis fungoides as a special species of skin sarcoma'.

In 1902 Brocq first used the term parapsoriasis to describe a heterogeneous group of conditions including plaque, lichenoid and guttate forms.[6] Many of the disorders included under this heading had been described previously and given separate names. Despite some clarification 50 years later by Civatte,[7] the term parapsoriasis continues to be used in different ways by different workers. Poikiloderma atrophicans vascularis was a term coined by Jacobi in 1908 to describe a particular clinical constellation of skin signs including pigmentary abnormalities, atrophy and telangiectasia.[8] Subsequent writers have considered the condition described by Jacobi to have been dermatomyositis, but the term poikiloderma was also used by Lane to describe a patient who later developed evidence of Hodgkin's disease.[9] One of Samman's major contributions many years later was the clinicopathological separation of the benign type of parapsoriasis from the 'pre-reticulotic' form of poikiloderma.[10]

The early part of the twentieth century saw no really major advances in our understanding of cutaneous lymphomas, although during this time Pautrier described the histopathological hallmark of mycosis fungoides which paved the way for modern scientific investigation into the relationship between the T lymphocyte and the epidermis. In 1911, Peyton Rous published his studies on the transmission of malignant tumours in mice by means of a cell-free infiltrate. This seminal paper was also to have important implications for our understanding of T cell lymphoma in man.

During the second quarter of this century further clinicopathological studies of varieties of cutaneous lymphoma were published. These included the syndrome of erythroderma, oedema, palmo-plantar keratoderma and lymphadenopathy associated with 'cellules monstreuses' in the circulating blood described by Sézary and Bouvrain in 1938,[11] and a report of a curious slowly growing plaque on the arm of a 13-year-old boy by Woringer and Kolopp in the following year.[12] It was, however, not until the late 1960s that real progress in understanding the fundamental nature of the various forms of cutaneous lymphoma was achieved. This was largely due to an unprecedented surge in research activity about this time in the fields of immunology and haematology and the correlation of this data with the studies of dermatologists and other clinicians. Advances in cutaneous lymphoma in the last 20 years can be considered under the headings of immunopathology and histogenesis, new clinicopathological variants of cutaneous lymphoma, and diagnosis and management.

IMMUNOPATHOLOGY AND HISTOGENESIS

It is salutory to realize that it was as recently as 1971 that the Sézary cell was first characterized as an abnormal lymphocyte,[13] although the ultrastructural morphology had been described 3 years previously by Lutzner and Jordan.[14] Soon afterwards, the T cell nature of the Sézary cell was reported by Brouet and the functional helper characteristic of the cell described by Broder.[15] At this time, the only widely used histopathological classification of lymphomas was that proposed by Rappaport in the mid-fifties based on the histopathology of affected lymph nodes. The scheme was found to be easily reproducible and clinically useful. The classification, however, took no account of the functional characteristics of the lymphoma cells, and a large number of the tumours classified under Rapapport's system as 'histiocytic' we now recognize as of lymphocyte lineage. The concept of lymphomas as tumours of the immune system and the recognition of B and T lymphocytes led to new systems of lymphoma classification devised by Lukes and Collins and the Kiel group in 1974. It should be noted at this point that cutaneous T cell lymphomas do not fit too neatly into any of the current histopathological classification systems, which were all originally based on studies of non-Hodgkin's lymphoma of lymph nodes. The revolutionary development of Kohler and Milstein in 1976 of the monoclonal hybridoma technique,[16] unleashed an exciting new era of investigation into cutaneous lymphoma. Extensive work in the early 1970s confirmed the T cell nature of mycosis fungoides and related conditions, although the requirement of frozen tissue for labelling studies with the first monoclonal antibodies limited the usefulness of the technique for routine diagnostic use. Initial eagerness for monoclonal antibody labelling techniques, and the hope that monoclonals would be found that would differentiate malignant neoplasms from reactive conditions in all cases of diagnostic difficulty, soon gave way to a more considered approach to their application. An appreciation of the technical problems involved, the realization that positive labelling is associated with, and not specific for, certain cell lines, and the discovery that tumour cells gain and lose antigenic determinants depending on the stage of disease, have all led to a better understanding of cutaneous lymphoma. Correlation of labelling techniques with light microscopic and ultrastructural findings has strengthened this understanding. We now know that what we have previously considered as mycosis fungoides is a very heterogeneous group of conditions. Not all skin lymphomas are T cell lymphomas and not all T cell lymphomas are of helper cell lineage. Moreover, we now know that tumour cells in some lymphomas show characteristics of various cell lines depending upon the technique used to study them. The appreciation of the immunohistological diversity of cutaneous lymphomas has helped to explain many of the clinical vagaries previously observed in this group of disorders. The availability, in recent years, of monoclonal antibodies which produce good results when used on paraffin embedded material,[17] and the development of a standard nomenclature (the cluster of differentiation or 'CD' system) for leukocyte membrane antigens,[18] have enabled the monoclonal antibody technique to be extended further in the field of diagnostic pathology.

Coincidental with, and related to, the interest in monoclonal antibodies, major advances were being made in the understanding of T cell ontogeny and function. Not only were different subsets of T cells becoming better defined, but the importance of the inter-relationship of these subsets with other lymphocytes, macrophages, antigen-presenting cells and keratinocytes began to be appreciated. The concept of an integrated system involving epidermal keratinocytes, epidermotropic T cells, Langerhans cells and peripheral lymph nodes (the SALT hypothesis) was put forward by Streilein in 1983. This idea, which suggested that the skin is an organ responsible for immunosurveillance against external allergens, supported several theories of the histogenesis of mycosis fungoides and gained wide (though not universal[19]) acceptance. There was, however, in the early eighties, still controversy regarding the nature of the pathological process occurring in mycosis fungoides. It was widely acknowledged that the disease ran a relatively chronic and benign course in a large number of cases, but that the condition could behave in a more aggressive manner in some patients, with rapid systemic dissemination of disease and death. Was mycosis fungoides a malignant disease from the outset, or did the early stages of the condition represent a reactive process which was only 'switched on' to behave in a malignant way in response to certain stimuli? This controversy remains largely unresolved today, although newer techniques, including cytogenetic and T cell receptor gene rearrangement studies, support the clinical view that at least advanced mycosis fungoides represents a clonal and malignant proliferation.

The precise aetiology of cutaneous T cell lymphoma is another area which, despite intensive study over the years, still eludes us. We have, however, a few clues; there is at least circumstantial evidence that exposure to environmental toxins of industrial origin may predispose some patients to the development of the disease. A genetic predisposition may also be important; certain HLA antigens are over-represented in patients with mycosis fungoides. However, perhaps the most exciting development of the last 10 years regarding the possible aetiology of mycosis fungoides relates to the role of viral infection. Until 8 years ago, infectious human retroviruses were unknown, and then in 1980 Poiesz and colleagues, working in Gallo's laboratories, first isolated HTLV-I from an American patient with what is now known as adult T cell leukaemia-lymphoma.[20] It is interesting to note that at the time of the report the patient was considered to be suffering from an aggressive form of mycosis fungoides. The association between the HTLV-I retrovirus and the form of T cell leukaemia-lymphoma endemic in parts of Japan and the Caribbean was soon made. Ultrastructural studies of immortalized cell lines from further patients with cutaneous T cell lymphoma resulted in the observation of retrovirus particles and the detection of reverse transcriptase activity. Evidence of seropositivity to HTLV-I in small numbers of patients with classical mycosis fungoides has recently been demonstrated, and a preliminary, but exciting, report from workers in Rome[21] suggests a new retrovirus, termed HTLV-5, may be associated with at least some cases of mycosis fungoides.

NEW CLINICOPATHOLOGICAL VARIANTS OF CUTANEOUS LYMPHOMA

Following the original clinical descriptions of the Alibert-Bazin variety of mycosis fungoides last century, numerous clinical varieties of the condition have been reported. Bullous, hyperkeratotic, granulomatous and hypopigmented forms of the condition are widely recognized and make accurate diagnosis at times very difficult for even the experienced clinician. In the 1970s progress in the understanding of lymphocyte function and immunology led to re-evaluation of some previously described forms of mycosis fungoides and the recognition that various forms of nodal non-Hodgkin's lymphoma could affect the skin. Thus, the entity of tumeur d'emblée already referred to, probably represents a varied group of malignancies including different forms of B cell and T cell neoplasms and probably also tumours of other cell lineage. Systemic T cell lymphomas that may involve the skin include the T lymphoblastic lymphomas that occur in older children, T cell lymphocytic and prolymphocytic leukaemia, T cell immunoblastic lymphoma and lympho-epithelioid (Lennert's) lymphoma. The clinical features of HTLV-I associated leukaemia-lymphoma are now well recognized; skin involvement is common as are hypercalcaemia, osteolytic bone lesions and a rapidly progressive course. It is interesting that the morphology of the atypical T cell associated with HTLV-I disease with its multilobar nucleus is somewhat similar to that described by van der Putte *et al.* in 1982 in a group of patients suffering from a localized non-epidermotropic form of T cell lymphoma.

Nosological confusion still remains today in relation to Sézary syndrome, in particular with regard to the criteria for diagnosis. Various workers use different criteria and this may explain some of the apparent anomalies found in case reports, particularly those discussing clinical responses to treatment regimens. In the Skin Tumour Unit at St. John's Hospital we use the criteria of erythroderma, pruritus and a circulating Sézary cell count of more than 1×10^9/l. These are arbitrary, but reproducible, criteria and we recognize that the group of patients so classified is a heterogeneous one. Many patients fulfilling these criteria will have a malignant T cell lymphoproliferative disorder which can perhaps be regarded as a 'leukaemic' form of mycosis fungoides. Yet other patients may have a form of chronic T lymphocytic (or prolymphocytic) leukaemia or HTLV-I associated disease. It must also be recognized that a small, but significant, group of patients with erythroderma and circulating Sézary cells may be suffering from benign reactive disorders such as erythrodermic eczema, drug reactions or photosensitivity. New investigational techniques, including T cell receptor gene rearrangement studies and determination of the functional sub-type of the predominant proliferating T cell,[22] may help to separate the patients with benign conditions from those with a genuine malignant lymphoproliferative disorder.

Reference has already been made to the clinical variant of cutaneous T cell disease described by Woringer and Kolopp and sometimes known as pagetoid reticulosis. The clinical features of a solitary plaque, often on an acral site, which gradually increases in size over a long period, and the epidermotropic histology, are well recognized. It now seems clear that both T lymphocytes (helper or suppressor or both) and cells of the histiocyte-macrophage series are involved in the pathogenesis of this disorder. Why the disease remains confined to one site over long periods of time in the vast majority of cases remains an enigma. Despite the relatively benign clinical course of this disorder, evidence has been recently published supporting its clonal nature.[23]

Another entity described in the last 15 years for which there is now evidence of clonal lymphocyte proliferation is granulomatous slack skin disease.[24] It is interesting to draw parallels with another atrophic skin disorder, acrodermatitis chronica atrophicans, in which cutaneous lymphoma may sometimes develop.

In his description of lymphomatoid papulosis in 1968, Macaulay emphasized the benign clinical course of the disease process which contrasts with the malignant-looking histological features. Investigations of the condition with modern cytogenetic techniques confirm that many cases of lymphomatoid papulosis are clonal in nature and that the cells in the skin may contain abnormal amounts of DNA. In recent years it has been recognized that a significant number of cases of lymphomatoid papulosis do, if followed up for long enough, eventually develop a T cell lymphoma, often of an aggressive type. Questions are now being asked about lymphomatoid papulosis that were asked many years ago about mycosis fungoides. Is the condition a malignant one from the start? If so, why is there such a chronic benign course in the majority of patients? The condition of regressing atypical histiocytosis described by Flynn in 1982 poses similar problems. It seems clear that T cells play an important role in the pathogenesis of the disorder, but the precise relationship of the condition to lymphomatoid papulosis and more 'classical' forms of cutaneous T cell lymphoma remains to be established.

MANAGEMENT

Correct management of any disorder involves the making of the diagnosis, investigations to establish the extent of the problem and the application of appropriate therapeutic modalities. The exciting advances in the last 20 years have made our task both easier and more difficult. Techniques such as the use of monoclonal antibodies in diagnostic pathology, DNA cytometry, cytogenetic and gene rearrangement studies and quantitative morphological microscopic measurement are valuable tools, but the pitfalls should be recognized and the results obtained carefully correlated with clinical findings. The development of staging systems for cutaneous lymphoma has enabled us to document disease progression more accurately. Early staging classifications proposed by various workers were, however, all slightly different and often not directly comparable. The TNM system devised by an American group, although not ideal, is a useful classification which has gained wide acceptance.[25] The concept that cutaneous T cell lymphoma is not a purely cutaneous disease has been acknowledged since the last century, but it was the post-mortem studies of Long and Mihm and Rappaport and Thomas in 1974 that first clearly demonstrated the extent of systemic involvement in patients dying with mycosis fungoides. The new investigational tools we have at our disposal have helped us detect early systemic involvement with cutaneous lymphoma, but in some cases the more closely we examine certain tissues, the more abnormalities we find. The significance of some of these abnormalities is then difficult to interpret. A particular problem is encountered, for example, with studies on lymph nodes. Is a node which shows slight expansion of the paracortex with small numbers of atypical T lymphocytes truly 'involved' with the lymphomatous process? If so, what implications does this have for the patient's prognosis or the choice of treatment regimen? These questions will only be answered by careful long-term follow-up of patients and controlled clinical trials.

What have we learnt since the times of Dr Funk regarding specific forms of therapy? The range of therapeutic modalities used by different groups varies widely. This is partly based on the clinician's concept of the nature of cutaneous T cell lymphoma; those who consider the disease to be a relatively benign one in the majority of cases are often reluctant to recommend aggressive treatment regimens in the early stages, whereas another school of thought suggests that early vigorous therapy is desirable in an attempt to eradicate what is a malignant and life-threatening disease. Whoever is right, it has become clear that over the years we have become quite efficient at controlling the symptoms and signs of cutaneous T cell lymphoma, at least on a temporary basis. It is less clear, even today, what effect the treatments we use have on patient survival. The usefulness of photochemotherapy, topical nitrogen mustard and related compounds, and othovoltage radiotherapy in controlling the cutaneous signs of disease is now well established. Photochemotherapy, although originally described for the treatment of mycosis fungoides in the United States, has been used in Britain since 1977 and at St. John's Hospital we have now treated over 200 patients with mycosis fungoides with PUVA. Electron beam therapy has also been used worldwide in a large number of patients. Our philosophy regarding this modality of treatment has changed over the years. In the early 1970s we used whole body electron beam (total dose 2000–3000 cGy) to treat patients with early stage disease in an attempt to cure the condition. Although the early response to treatment was encouraging, it soon became clear that in a large number of patients the disease relapsed after a period of 2 to 4 years. We now reserve treatment with whole body electrons for patients with more advanced disease whose disease is not controlled by PUVA or topical nitrogen mustard supplemented by local orthovoltage radiotherapy.

Recently, the techniques of leukapheresis and photopheresis[26] have shown some promise in the management of cutaneous T cell lymphoma. These regimens may prove to be particularly useful in the treatment of patients with Sézary syndrome—a condition which is usually more difficult to manage than the 'classical' form of mycosis fungoides.

Multiagent chemotherapy is usually reserved for advanced disease, and it is widely recognized that the majority of cutaneous T cell lymphomas respond much less predictably than cutaneous lymphomas of B cell type. The development of the retinoid group of drugs has added another useful weapon to our therapeutic armametarium. Some patients with T cell lymphoma seem to respond well to retinoids, but it is difficult to predict which patients will do well on this regimen.

In the last few years, exciting new agents have become available, which, because of their effects on various components of the immune system, suggest that, at least on theoretical grounds, they may have a useful clinical role in the treatment of cutaneous lymphoma. These drugs include cyclosporin A, the interferons and interleukins. Other so-called biological response modifiers are sure to follow. The experimental use of monoclonal antibodies raised against specific tumour cell lines has opened up further therapeutic horizons. It is hoped that further experience with these newer agents and the better definition of the different clinicopathological subsets of cutaneous T cell lymphoma will soon lead to a more rational approach to therapy of this most challenging and complex group of disorders. It is perhaps heartening to learn that some 100 years ago management of mycosis fungoides was also difficult. Dr Funk of Warsaw noted that the condition 'ends almost regularly fatally' and that the only reported cure was 'from six to twenty-five drops of Fowler's solution daily'. I am looking forward to the next 100 years.

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Centenary Review

The control of infective skin diseases—the lessons of leprosy research

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One of the major changes seen in the past 100 years has been a shift in emphasis away from infections as common causes of skin disease in non-tropical countries. In 1888, dermatoses thought to be caused by, or secondary to, syphilis were common in everyday dermatological practice, and papers on this condition and its treatment were issues regularly aired in the medical literature. The first edition of the *British Journal of Dermatology*, for instance, contains a paper by Unna, on the association between seborrhoeic eczema and syphilis.[1] Cutaneous forms of other infectious conditions, such as tuberculosis, as well as endemic scalp ringworm were prevalent in dermatological out-patient clinics. Since then there has been a decline in the importance of infections as causes of skin disease, largely through the discovery of antibiotics and improvements in social conditions, although this picture is likely to alter with the emergence of the acquired immunodeficiency syndrome which threatens to dominate the pattern of disease for years to come. By contrast, infections are still major causes of skin, as well as internal, disease in the tropics, particularly in rural communities. In recognition of the possibility of eliminating the important transmissible diseases through concerted effort, the World Health Organization has designated six infectious causes of disease as targets for a special programme of tropical disease research. These were malaria, schistosomiasis, trypanosomiasis, filariasis, leishmaniasis and leprosy. While the latter three conditions feature prominently as causes of skin disease in the developing world, the advances, as well as the setbacks, in infectious disease research over the last century are perhaps best illustrated by the only bacterial infection in this group—leprosy.

HANSEN'S DISEASE

Leprosy or Hansen's disease is one of the oldest diseases known to mankind. It principally affects the skin, peripheral nerves and mucous membranes of the upper respiratory tract. There is evidence of its existence many centuries ago in ancient Indian and Chinese writings. It was endemic in Europe up to and throughout the Middle Ages, but its prevalence declined thereafter. In the nineteenth century only pockets of the disease were still found in northern Europe including Norway. In southern Europe decline of the infection has been slower and in the tropics, from Latin America to Africa and the Far East, leprosy remains a major public health problem.

The year 1888 has no particular significance in the history of leprosy. A decade earlier, in 1873, Armauer Hansen working in Bergen, Norway, had first demonstrated leprosy bacilli in smears taken from the lesions of patients with leprosy and, although he had experienced considerable difficulty in staining the organisms, his findings are widely accepted as the first demonstration of the true cause of the disease.[2] Perhaps 1888 should be remembered as the mid-point of an experiment, unthinkable in current medical ethical practice, in which a condemned murderer called Keanu in the Hawaian islands was inoculated with material from a leprous lesion. By 1890 he had developed confirmed leprosy and died in 1894 thus demonstrating direct transmission of the disease.[3] It was many years before an acceptable animal model could be found which would confirm the transmissibility of leprosy.

Although the different clinical manifestations of leprosy were well recognized by the nineteenth century, the relationship between one form and another was still a mystery. In addition, there was no effective therapy or adequate surveillance. Much of the progress in leprosy research since then has been concerned with understanding the pathogenesis of the disease and the development of appropriate treatment and control measures. As with other infections, great advances have been made in our understanding of the different clinical forms of leprosy, the structure and chemical composition of the organism, the host's immune response and the most appropriate methods of therapy.

THE CLINICAL SPECTRUM

It had been known for many years that patients with leprosy presented with a variety of clinical patterns ranging from diffuse infiltration of the skin and other organ systems by large numbers of bacillus-laden macrophages which produced characteristic infiltrative lesions, to large irregularly distributed plaques, showing a degree of anaesthesia, which on biopsy contained few bacilli, but well-ordered granulomas involving dermal structures. The conclusion that this represented a spectrum of reactions determined by the patient's immune responses, was proposed by Ridley and Jopling as the basis for a new classification of the disease.[4] At one pole, represented by tuberculoid leprosy (TT), few organisms were present and there was a pronounced cellular immune response with resulting neurological damage. At the opposite pole, lepromatous leprosy (LL), immune responsiveness was poor and large numbers of bacilli present. Intermediate stages representing borderline lepromatous (BL) or borderline tuberculoid (BT) forms or borderline leprosy (BB) showed features somewhere between the two extremes. The Ridley Jopling classification has proved of considerable value both in the management of leprosy and in furthering our understanding of the pathogenetic mechanisms involved, although there are alternative schemes including a clinical classification (Madrid) and one prepared by the Association of Indian Leprologists, both of which are also widely used.

In addition to the more stable forms of leprosy, a number of reactive states or reactions have been described which appear to follow changes in the patient's immunological perception of the infection. Type 1 reactions, for instance, occur when a patient has either an increase (reversal) or decrease (downgrading) of immunity. Type 2 reactions, such as erythema nodosum leprosum, occur with LL and BL patients and are thought to represent either deposition of immune complexes or T lymphocyte-mediated processes such as an increase in activities related to antigen recognition by T lymphocytes.

In the last 100 years, one unusual form of leprosy reaction was 'rediscovered'. Lucio's disease, which is seen most frequently in Mexico, is characterized by the appearance of large irregular haemorrhagic infarcts of the skin. It was originally described in 1852 by Lucio and Alvarado in a treatise on leprosy in which they classified the clinical forms into three varieties—anaesthetic (tuberculoid), nodular (lepromatous) and a third form called 'manchada' meaning spotted.[5] The original report also contained details of the other features of the condition such as loss of eyebrows secondary to diffuse lepromatous infiltration and impaired sensation. Lucio's leprosy was largely ignored until Latapi drew attention to its existence in Mexico in 1938[6] and since then it has been seen regularly in Latin America and the U.S.A. The pathogenesis of the condition is not entirely understood, although vascular endothelial cells in the dermis appear to be packed with bacilli. Patients with Lucio's leprosy also have an early phase (4–6 h) reaction to intradermal lepromin antigen, culminating in central necrosis.

Reactive states are not unique to leprosy and are now familiar occurrences in other infective processes from viral to fungal infections. They reflect the capacity of the body's immune system to change during an infection and participate in events which may eventually contribute to the disease moribidity.

MYCOBACTERIUM LEPRAE—STRUCTURE AND ANTIGENIC COMPOSITION

A major phase in the history of the study of infections during the last century has been the development of techniques for studying micro-organisms outside the host. Many bacteria and fungi will grow on simple defined laboratory media, while viruses generally require the more complex environment provided by cell culture. However, a number of organisms, *Mycobacterium leprae* amongst them, have proved difficult or impossible to maintain under laboratory conditions and at present there is no reproducible method for growing the leprosy bacillus in culture.

Numerous attempts have also been made to reproduce the disease in animals, but none were successful until Shepard showed that was possible to induce a limited form of infection in the foot pad of the mouse.[7] This observation paved the way to a number of investigations ranging from studies of the generation time of bacilli to the testing of drugs and antibiotic sensitivities. The thymectomized and irradiated mouse also proved to be a model of lepromatous disease, blood stream dissemination occurring in infected animals.[8] A more natural host was provided by the nine-banded armadillo, which proved uniquely susceptible to *M. leprae*, probably because of its low body core temperature.[9] In nature, leprosy has been detected rarely in a number of animals such as the chimpanzee, the Mangabey monkey and the nine-banded armadillo itself.

As with other infections, such as those caused by the human papilloma viruses, a number of recent experimental techniques have come to the aid of those working with organisms which do not replicate in laboratory culture. These range from studies of cell ultrastructure to the use of monoclonal antibodies and molecular genetics. The latter may be used to uncover specific antigenic and genetic features of the organism. The *M. leprae* cell is surrounded by a capsule composed of phthiocerol and an important antigenic constituent, phenolic glycolipid. Internal to this is a two-layered cell wall and the plasma membrane. The cell cytoplasm is also the source of a number of dominant antigens.[10] Several investigators have successfully produced monoclonal antibodies of different specificities to *M. leprae*; one showing potential promise is directed against a component of the phenolic glycolipid capsule. The latter contains a terminal trisaccharide which appears to be unique to *M. leprae*.[11] However, early attempts to raise monoclonals to this component resulted in antibodies which cross-reacted with other mycobacteria, suggesting common epitopes or non-specific reactivity.[12] An alternative candidate specific antigen is a protein component of molecular weight 65 kd. In a recent study comparing different monoclonals to this antigen it was found that there are 14 recognized distinct antigenic epitopes on this protein, but of these one recognized an antigenic site only found in *M. leprae*.[13]

The discovery that it was possible to transplant DNA from one organism into plasmid or genomic DNA of another, so that the new 'vector' would express the relevant characters of the donor, was a revolutionary advance in medicine. The application of this technique to leprosy research is currently the subject of considerable study. It has been shown that DNA isolated from *M. leprae* and encorporated into the genome of the λgt 11 bacteriophage may encode antigens of defined molecular weight and recognized by different monoclonal antibodies.[14] This is an important first step in producing antigens with immunological activity, including possible vaccines. Genetically engineered vaccines have considerable potential in the control of infective conditions, provided it is possible to maintain immunological activity while refining specificity.

SUSCEPTIBILITY AND HOST RESISTANCE

Patients vary in their ability to defend themselves against most infections, an idea implicit in the Ridley Jopling classification of leprosy. However, the factors underlying transmission and individual susceptibility to the latter are incompletely understood. While many early workers thought that contamination from skin lesions was the most likely route for transmission of leprosy, the importance of the excretion of large numbers of organisms from the nose as a potential source was recognized as long ago as 1898.[15] There are a number of different hypotheses which may account for variations in subsequent susceptibility. Firstly, it has been suggested that susceptibility to the different clinical forms of leprosy is genetically mediated. Some support for this view has come from the demonstration of an association between HLA-DR2 and tuberculoid leprosy.[16] An alternative possibility is that susceptibility is determined by previous exposure to other (environmental) mycobacteria.[17] The latter are known to have the capacity, *in vitro*, to affect expression of immune responses; exposure to different mycobacteria also varies considerably in different geographical areas. Other authors have suggested alternative hypotheses. For instance, a recent study of leprosy in Tamil Nadu province in India showed a correlation between malnutrition in children aged 1–4 and the occurrence of leprosy.[18] While at present it is not possible to produce a universally satisfactory theory to account for all these observations, it is quite possible that a number of different factors are implicated in determining the outcome of exposure to infection in a genetically predisposed individual.

After infection, a patient's immunity to *M. leprae* is determined by T lymphocyte and macrophage mediated mechanisms. The evidence for this is largely based on the correlation between paucibacillary (tuberculoid) forms of leprosy and the presence of activated lymphocytes and delayed type hypersensitivity to mycobacterial and heterologous antigens.[19] However, the investigation of immunity in leprosy patients has only been possible relatively recently and progress was hampered by the absence of a satisfactory animal model. In lepromatous leprosy there is either a primary or secondary defect in the development of effective T cell responses. As with other infections, the defective reactions were initially thought to represent innate immune defects. However, attention has focussed more recently on immunoregulation by antigenic components of *M. leprae* itself.

The mechanisms of immunological regulation in leprosy have been partially characterized. The lymphocyte infiltrate in tuberculoid forms shows an excess of helper/inducer (OKT4) cells over suppressor/cytotoxic (OKT8) cells, in an approximate ratio of 2:1. The reverse is true in lepromatous forms. Peripheral blood mononuclear cells from patients with LL exposed to lepromin show suppressed blastogenic responses to concanavalin A, mediated via OKT8 cells. This is thought to be initiated via the terminal disaccharide of the phenolic glycolipid. Another suppressor antigen is a lipoarabinomannan, a cell wall constituent. The presence of lymphocytes bearing OKT8 markers has been demonstrated by the ingenious method of removing cells from infiltrates in skin biopsies and then inducing clonal expansion of extracted cells in tissue culture.[20] Such cells may predominate in infiltrates from lepromatous, but not tuberculoid leprosy skin. In addition, lesions of LL are infiltrated with fewer cells producing and accepting interleukin 2 (IL-2) compared with tuberculoid lesions.[21] Conversely, helper lymphocytes in lepromatous patients may show defective responses on antigenic stimulation. Other regulatory mechanisms have been associated with mycobacteria such as the production of anti-idiotype antibodies which, by binding to T lymphocytes, affect the expression of immune responses.[22]

The finding that many micro-organisms may influence the expression of immune reactions has had a profound effect on the interpretation of immunological tests which were previously thought to indicate a primary immune defect. It does, however, mean that the use of immunologically active compounds, including cytokines such as IL-2 and γ-interferon, in the treatment of these conditions is a logical development.

ANTIBIOTICS AND LEPROSY

One of the first major steps in the control of leprosy was the discovery that sulphone drugs and later rifampicin, ethionamide and clofazimine were clinically active in leprosy. While 4,4′ diaminodiphenyl sulphone (DDS or dapsone) was first synthesized in Germany in 1908 by Fromm and Whitman[23] it was not until 25 years later that workers in London and France reported the effects of the drug in streptococcal infections of mice. This paved the way for the first studies of dapsone in the U.S. Leprosarium in Carville, Louisiana in 1943. The efficacy of the drug was quickly established and it became the staple of treatment regimens. Control programmes were organized and the elimination of the disease by chemotherapy seemed possible. However, by the early 1950s it was widely suspected that dapsone resistance was a potential hazard, although the first proven case was only established in 1964 using the mouse foot model. The first case reported in the United Kingdom was described by Adams and Waters in 1966.[24] The patient had an initial good response to dapsone and thiambutosine, but 9 months after the latter was stopped, developed small nodules over the body. The organisms from these lesions were found to be dapsone resistant in the mouse foot pad test.

Primary dapsone resistance (drug resistance demonstrable before the start of treatment) while initially rare, is becoming more prevalent. Secondary dapsone resistance developing during therapy is more common. In 1981, for instance, it was estimated that in at least 10% of cases receiving dapsone monotherapy in Malaysia the organisms were resistant *in vitro* to the drug.[25] Drug resistance is not confined to dapsone and both rifampicin and ethionamide resistant isolates have been found. At present there have been no proven cases of clofazimine resistance. However, as with other mycobacterial infections, rapid destruction of organisms is possible in leprosy. For instance, with rifampicin 99·99% of *M. leprae* appear to be non-viable within 1 week of starting therapy.

Taking these arguments into consideration, in 1981 a special committee of the World Health Organization produced guidelines for leprosy chemotherapy in developing countries.[25] The essentials of their recommendations are shown below:

For multibacillary leprosy (LL, BL, BB): dapsone 100 mg daily plus rifampicin 600 mg monthly and clofazimine 50 mg daily and 300 mg monthly. The intermittent use of rifampicin is justified because of its rapid cidal activity, discussed previously, coupled with the slow multiplication rate of *M. leprae*.

For paucibacillary leprosy (I, TT, BT): dapsone 100 mg daily and rifampicin 600 mg once a month for 6 months.

The committee warned that it was not possible to predict the relapse rate, and indeed the recommendations are not universally followed. It was none the less an important attempt to strike a balance between the ideal and the practicable. It is also an example of a more recent development in the history of antibiotics—the formulation of guidelines for the optimum use of such drugs.

THE LEPROSY VACCINE

While chemotherapy is an effective method of treatment, immunization has remained an important target in leprosy control as it is more widely applicable to large populations. The whole field was given an important stimulus in 1939 by the work of Fernandez in Argentina, who observed that children receiving BCG as part of a tuberculosis programme converted to lepromin positivity.[26] This finding was quickly confirmed in a number of different countries, providing the stimulus to assess the value of BCG as a leprosy vaccine. It was used as an immunizing agent in a variety of areas, but with varying degrees of success. For instance, protection rates of 81% 10 years after immunization with BCG have been seen in Uganda. In other countries such as India, immunization with BCG was only protective in 26% of those immunized. A recent study in Burma produced similar somewhat disappointing results and showed that the ability to confer protection varied with a number of different factors including the batch of BCG and the age of the patient at immunization.[27]

While BCG is still a possible candidate vaccine there are alternatives. For instance, killed *M. leprae* bacilli have been used in mice and can produce moderate degrees of protection. Another group have shown that a mixed vaccine consisting of *M. leprae* and BCG may offer better protection,[28] although it may produce a high frequency of skin reactions in humans after injection.

Given that the antigens of *M. leprae* are complex and show both immunostimulatory as well as inhibitory properties, considerable effort has been expended on the development of a genetically manufactured antigen composed of those components most suitable for effective immunization. Some of the earlier studies have been described above. With the recent discovery of the precise gene sequence of elements of one key *M. leprae* antigen (65 kd) it has been possible to synthesize peptides of 12–13 amino acid residues which determine the immunogenicity of such antigens.[29] The application of these synthetic peptides to leprosy immunization is in progress.

CONCLUSION

Infectious disease research has covered much ground in 100 years. In 1888 the first attempts to isolate and categorize infectious organisms were being made, but in many cases it had not been possible to establish their involvement in the disease process by objective criteria. Methods for laboratory diagnosis and treatment were necessarily crude. In the succeeding years progress has been rapid, particularly in the fields of diagnostic microbiology, chemotherapy and immunology. The ability to sequence sections of the genome of many organisms is the latest, and perhaps most remarkable, of these achievements. The progress in our understanding of one of the oldest known infections, leprosy, amply illustrates the extent of these advances.

Although it is an important example, leprosy displays a number of unusual features. Firstly, it has not been possible to maintain the bacterium in laboratory culture, a problem seen with other skin pathogens from human papilloma viruses to the fungus *Loboa loboi*. The new technology of molecular genetics has proved an invaluable tool in circumventing this problem. Secondly, *M.*

leprae parasitizes Schwann cells, amongst other sites, and is almost certainly afforded some protection there by from immune surveillance. The natural history of the disease in also incompletely understood. Little is known, for instance, about the exposed individual who does not develop the disease. There have been reports that bacilli can be found in uninvolved skin in subjects who never develop any of the features of leprosy.[30] The status of these individuals and the conditions which determine progress to the polar variants of leprosy need to be defined. The advent of new serological tests which utilize specific antigens[11] may provide some of the answers.

While much of this account has dealt with clinical and laboratory aspects of leprosy, subsequent progress towards the elimination of the disease is largely dependent on the ability of countries in the endemic areas to deliver therapeutic measures and to monitor their short- and long-term effects. That such measures are necessary is well illustrated by the example of another infection principally affecting the skin, yaws, which was the subject of a worldwide eradication programme in the early 1960s. In the last few years, the disease has re-emerged in West Africa and the West Pacific. While the exact reasons are not clear, discontinuation of surveillance in the face of other health priorities is a likely explanation. The process of recruitment and maintenance of an infrastructure for disease eradication is both costly and time consuming. Unfortunately, without it control of the major endemic infections is not possible, even though the scientific goals are probably achievable within the forseeable future.

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Original Reports

Growth of melanocytes in a skin equivalent model *in vitro*

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SUMMARY

We have developed a pigmented human skin equivalent by inserting a punch biopsy of human infant foreskin as a source of epidermis into a collagen lattice (dermal equivalent). Using a conventional epidermal culture medium and stimulation with UVB irradiation or 8-MOP+UVA treatment, melanocytes were found to grow out from the biopsy with the epidermal sheet. In this newly formed epidermis, melanocytes and keratinocytes were maintained in an architectural relationship similar to that present *in vivo* and melanocyte outgrowth could be quantitatively evaluated.

Consequently, this pigmented human skin equivalent is a useful model for investigating the biology and photobiology of human skin pigmentation.

Considering the advantages of both three-dimensional cell and organ cultures,[1] we have recently developed a new dynamic culture system which allows the reconstruction of a living skin equivalent *in vitro*.[2] One possible application of this technique is to study both qualitatively and quantitatively the growth of a sheet of multilayered keratinocytes on a dermal equivalent. Keratinocytes originate from the margin of a punch biopsy implanted in the centre of the dermal equivalent and give rise to an epidermal sheet which progressively covers the dermal equivalent. As reported by Topol *et al.*,[3] melanocytes present in the punch biopsy, which gives rise to the new epidermis, do not spontaneously migrate with the keratinocytes.

In the present investigation, we used punch biopsy specimens of foreskin from patients of different ages, implanted the specimens into a dermal equivalent and stimulated the melanocytes with 8-MOP+UVA or UVB treatment, in order to induce the outgrowth of melanocytes from the implanted punch biopsy into the epidermal sheet. This *in vitro* model allows for qualitative and quantitative studies in skin pigmentation to be performed in a situation similar to that present *in vivo*.

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METHODS

Subjects

Specimens of human foreskin were obtained from five patients, aged 8 and 15 months and 4, 5 and 6 years, respectively, undergoing surgery for phimosis.

Dermal equivalent

Fibroblast cultures from the human foreskins were used to prepare dermal equivalents. Fibroblasts from confluent or subconfluent cultures removed by trypsin treatment, were suspended at 4×10^5 cells/ml in Earle's modified Eagle's medium (EMEM, Flow Labs) containing 10% fetal calf serum, 100 IU/ml penicilin, 100 μg/ml streptomycin (Flow Labs) and 2·5 μg/ml amphotericin B (Squibb). The dermal equivalents were prepared as previously described.[2] Briefly, 4·6 ml of concentrated EMEM with antibiotics, 0·9 ml fetal calf serum, 3·0 ml collagen solution (2 mg/ml in 1 : 100 acetic acid solution, extracted from rat tail tendons), 0·5 ml 0·1 M NaOH and 1·0 ml fibroblast suspension were mixed together and quickly poured into 60 mm diameter bacteriological petri dishes. The dishes were placed at 37°C in a 5% CO_2 atmosphere.

Epidermalization

Two millimetre punch biopsies were taken from the foreskins and each biopsy inserted, epidermis up, into the centre of a freshly cast dermal equivalent. As a result of the interaction between fibroblasts and collagen fibrils, gel contraction allows the implanted biopsy to remain firmly in place.

After 24 h, the culture medium released from the contracted dermal equivalent was renewed by adding an equal volume of EMEM supplemented with antibiotics, 10% fetal calf serum, 20 ng/ml of EGF (collaborative Research), 0·4 μg/ml of hydrocortisone (Roussel) and cholera toxin at a final concentration of 10^{-10} M (Sigma). The culture medium was changed 3 times a week.

At 5 days, the dermal equivalents were placed on stainless steel grids and maintained for 15 min without medium in the culture dishes to allow adherence to the grid which stops contraction. The dermal equivalents were then covered with 5 ml of fresh medium, and maintained at 37°C in a 5% CO_2 atmosphere.

Irradiation protocol

8-MOP + UVA treatment. UVA irradiation was performed using a Waldmann PUVA 200 unit (H.Waldmann GMBH, Werk für Lichttechnik Schwennigen, FRG) equipped with Sylvania F8T5 PUVA fluorescent bulbs, which emit broad band UVA from 320–400 nm, with a maximum at 360 nm. Fluence was determined using a VLX-365 nm radiometer (Wilber Lourmat, 77202 Marne La Vallée, France) and was found to be 2·7 mW/cm^2.

Immediately prior to UVA irradiation the culture medium was removed and the skin equivalents rinsed with Hank's medium (without phenol red). Five millilitres of a freshly prepared solution of 8-MOP (Sigma)(5×10^{-7} M in Hank's medium) was added. The 8-MOP concentration was the same as the mean level found in suction blister fluid of patients 2 h after oral 8-MOP ingestion and immediately prior to irradiation.[4] The skin equivalents were placed at 37°C for 60 min and just prior to UVA irradiation the 8-MOP solution was removed. The UVA dose chosen was in the range used *in vivo* for PUVA therapy and for melanocyte stimulation in animal model systems.[5,6] The skin equivalents were irradiated in a covered petri dish for 180 s which corresponds to 500 mJ/cm^2.

UVB irradiation. UVB irradiation was performed with a 2·5 kW Xenon lamp in a Kratos lamphouse equipped with a 10 cm water filter. The 313 nm light was obtained at the output of a Kratos GM 252 monochromator. The entrance and exit slits of the monochromator were both set at 2·3 mm leading to a 7·5 nm bandwidth. The fluence using a VLX-312 radiometer (Wilber Lourmat) was found to be 1·15 mW/cm^2. The culture medium was removed before irradiation to prevent the formation of toxic UV photoproducts in the medium. The UVB dose chosen was that used for melanocyte stimulation in animals, and cell and organ cultures.[1,3] The reconstructed skin equivalents were irradiated in covered petri dishes for 180 s which corresponds to 200 mJ/cm^2.

UVA or UVB irradiation was performed at 7 days and thereafter every 2 days (five irradiations in total). Controls were Hank's medium without irradiation (C), 8-MOP without irradiation (8-MOP) and Hank's medium with UVA irradiation (UVA). All assays were carried out in duplicate or triplicate. All cultures, both irradiated or non-irradiated, were rinsed with Hank's medium and refed with 5 ml of fresh epidermal culture medium. The experiment was stopped on the 17th day.

At the end of the experiment, the skin equivalents were separated into two sets; one set was examined for the dopa reaction, and the other processed for light and electron microscopy.

Dopa reaction

The epidermal outgrowth and the contiguous donor epidermis (from the foreskin punch biopsy) were removed with forceps and transferred to 35 mm petri dishes containing 10% formol saline for 15 min and then rinsed with PBS. They were then incubated in 0·1 M sodium phosphate buffer (pH 7·3–7·4) containing 0·1% dopa (L 3,4 dihydroxyphenylalanine, Sigma) for 4 h at 37°C in the dark. After 1 h the solution was removed and a fresh dopa solution was added. Then, the specimens were washed with PBS for 10 min and the epidermal sheets were then fixed in 10% neutral formalin overnight. The epidermis was dehydrated in alcohol and placed upside down on a glass slide, covered with a glass coverslip and sealed under glycerol.

Brownish-black dopa stained cells were scanned at × 400 magnification to distinguish between dendritic dopa-positive melanocytes and round dopa-positive cells. The newly formed epidermis was divided into radial zones extending from the central punch (Fig. 1) In each radial zone, the dopa-positive cells were counted in successive areas (0·25mm^2) from the punch biopsy to the outer margin of the new epidermis (maximum number of areas = 16). These areas were grouped in fours into fields (F), according to their distance from the biopsy (F1: the areas around the punch; F2 and F3: intermediate areas and F4: at the periphery). Cell counts were performed in duplicate or triplicate. The results were expressed as the mean number (± SEM) of dendritic dopa-positive melanocytes or total dopa-positive cells (dendritic + round) per radial zone.

Light and electron microscopy

For examination of semi-thin and ultra-thin sections, skin equivalents were fixed with Karnovsky's medium, post fixed, dehydrated, embedded in epon and sectioned as previously described.[8]

RESULTS

No difference in colour was observed between the irradiated dermal equivalents and the controls. Treatment with 8-MOP + UVA or UVB did not modify the surface of the epidermal outgrowths when compared with the controls.

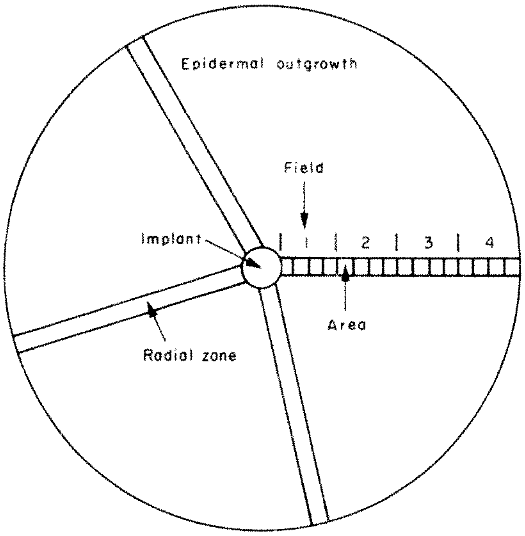

FIGURE 1. Diagram showing the fields in which dopa-positive cells were counted in the newly formed epidermis.

Quantitative studies of melanocyte outgrowth

The dopa reaction was performed on samples from all five patients and the initial density of melanocytes was found to be similar in all punch biopsies examined (250–350/mm^2). After 17 days of culture, dopa-positive cells were still present in the epidermis of the explants from all donors, but were less numerous than initially (about 100–200/mm^2) (Fig. 2A).

At the end of the experiment, we observed dopa-positive cells scattered in the newly formed epidermis from the 8 and 15 month-old donors. In contrast, in the new epidermis derived from foreskin biopsies from 4, 5 and 6 year-old donors dopa-positive cells were only rarely observed, even after stimulation with 8-MOP + UVA or UVB, and were located exclusively around the skin biopsy margin.

The dopa reaction revealed two types of brownish-black elements in both irradiated (8-MOP + UVA or UVB) and control epidermal outgrowths: dendritic dopa-positive melanocytes, and round dopa-positive cells (Fig. 2A and B). Even after UV stimulation, the dopa-positive cells in the epidermal sheet remained separated from each other as observed *in vivo*, but at a lower density.

The melanocyte outgrowth obtained under stimulation (8-MOP + UVA or UVB) was identical in the epidermal sheets derived from the 8 and 15 month-old donors. The quantitative results obtained from the 15 month-old donor are shown in Table 1 and Figure 3.

Distribution of the dopa-positive cells in the newly formed epidermis

The results are shown in Table 1.

The dopa-positive cells were more numerous around the punch (F1) than in other fields and their density decreased progressively towards the periphery (F4). In contrast to controls and to 8-MOP + UVA-treated specimens, after UVB irradiation dopa-positive cells were detected throughout the newly formed epidermis as far as the outer margin (F4).

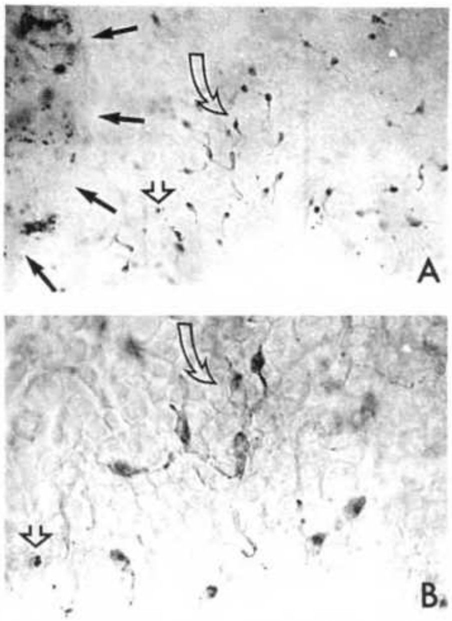

FIGURE 2. Dopa reaction on an epidermal sheet obtained from a 15 month-old donor, after 8-MOP + UVA treatment. (A) Edge of punch biopsy (←). Dopa-positive elements can be seen on the surface. Dopa-positive dendritic (⇨) and round cells (⇩) are scattered in the newly formed epidermis. (Original × 135). (B) Higher magnification of part of the newly formed epidermis showing dopa positive dendritic (⇨) and round cells (⇩) (original × 330).

TABLE 1. Distribution of dopa-positive cells in newly formed epidermis according to their distance from the punch biopsy

	Field			
Treatments	F1 Around punch	F2 Intermediate area	F3 Intermediate area	F4 Periphery
Hanks alone	+	+	○	○
UVA	+ +	+	+ rare	○
8-MOP	+ + +	+ +	+	○
8-MOP + UVA	+ + +	+ + +	+	○
UVB	+ + + +	+ + +	+ +	+

○: no cells; +: 0–5 cells/field; + +: 5–10 cells/field; + + +: 10–20 cells/field; + + + +: > 20 cells/field.

Dopa-positive cells/radial zone

The results are shown in Figure 3.

Dendritic melanocyte density was higher in newly formed skin equivalents treated with 8-MOP alone, 8-MOP + UVA or UVB than in the controls treated with Hank's alone or UVA alone.

Density of dopa-positive cells (dendritic + round) was increased in specimens treated with 8-MOP, 8-MOP + UVA or UVB compared with those treated with Hank's alone or UVA alone.

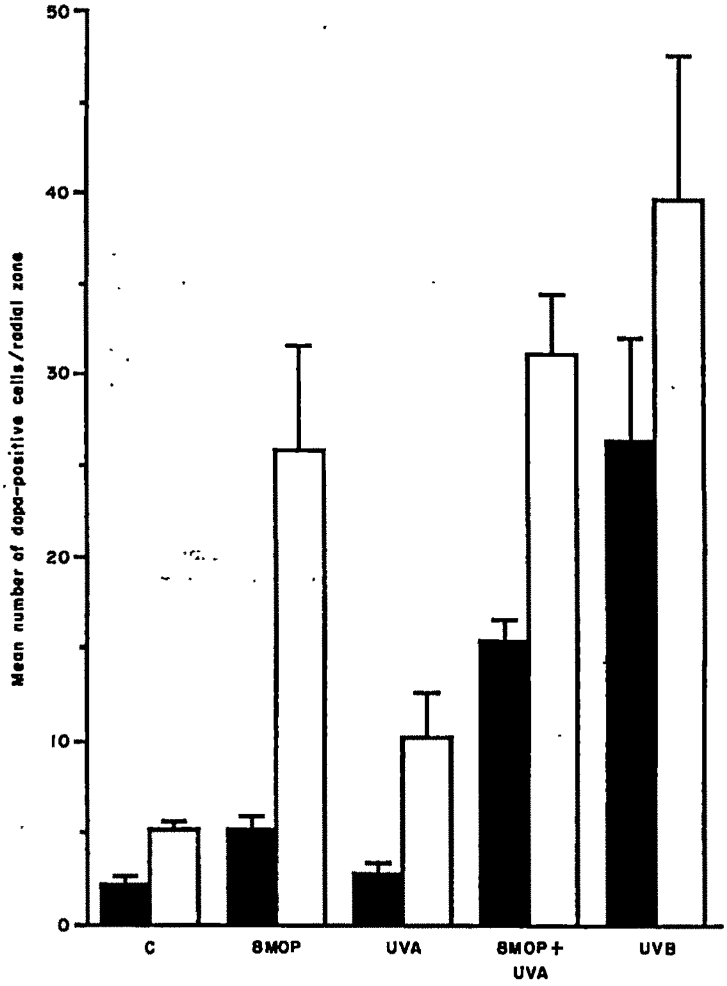

FIGURE 3. Effect of 8-MOP + UVA treatment or UVB irradiation on the density of dopa-positive cells in newly formed epidermis from a biopsy from a 15 month-old donor. Results are the mean numbers (± SEM) of dendritic (■) or total (□) (round + dendritic) dopa-positive cells per radial zone extending from the central punch. C = control in Hank's medium.

Round dopa-positive cells were increased not only with 8-MOP + UVA (15·80 ± 3·70 cells/zone) and UVB (13·20 ± 1·90), but also with 8-MOP alone (20·70 ± 4·50) as compared with UVA alone (7·45 ± 1·90) and Hank's medium alone (3·00 ± 0·35).

Morphological studies

Semi-thin sections. Semi-thin sections of 8-MOP + UVA or UVB-treated skin equivalents showed a differentiated multilayered epidermis containing melanocytes with a clear cytoplasm. These melanocytes were found in the basal layer (Fig. 4). Keratinocytes were more or less cuboidal in the basal layer. Higher in the epidermis the keratinocytes became progressively flattened, nuclei were no longer visible and a cornified layer was formed that desquamated.[2]

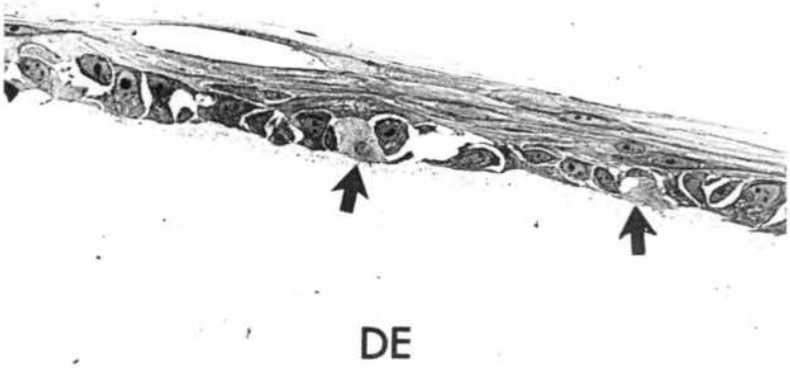

FIGURE 4. 1 μm section of 8-MOP + UVA-treated newly formed human skin from a biopsy from a 15 month-old donor. Above the dermal equivalent (**DE**) the differentiated multilayered new epidermis can be seen. Melanocytes (➔) are identifiable by their clear cytoplasm Note their basal location. (Original × 630).

In 2 μm sections of newly formed skin, pigment granules were seen in melanocytes and in the neighbouring keratinocytes at several levels of the epidermis. No evidence of cellular necrosis was observed in irradiated newly formed skin.

No melanocytes were observed in the dermal portion of the skin equivalent in irradiated or non-irradiated specimens.

Thin sections. Electron microscopy of the clear cells observed in semi-thin sections demonstrated that they were characteristic melanocytes (Fig. 5). These cells showed neither desmosomes nor tonofilaments. Vesicles, premelanosomes and melanosomes with different degrees of melanization (i.e. granules of various electron densities) were seen in the melanocytic perikaryon.

DISCUSSION

The present study shows that human melanocytes behave differently to keratinocytes in a dynamic organ culture system. During a 17-day period of culture, there was an outgrowth of keratinocytes from punch biopsies similar to that seen in wound healing. Melanocytes were able to grow into the epidermal sheet only in response to physical or chemical stimulation. These results are in agreement with the fact that melanocytes are cells with a low mitotic index *in vivo*[9] as well as *in vitro*.[10] Moreover, in culture normal melanocytes grow only in the presence of potent growth promoting agents or in the presence of natural mitogenic factors for melanocytes found in various tissue extracts such as hypothalamic bovine extract[11] or in human placenta.[12] However, these growth factors promote the growth of melanocytes only in the presence of cyclic-adenosine-monophosphate (cAMP) stimulators.[13] Agents such as tumour promotors or antimetabolites, are toxic for keratinocytes.[14-16] In a skin equivalent model, we were able to

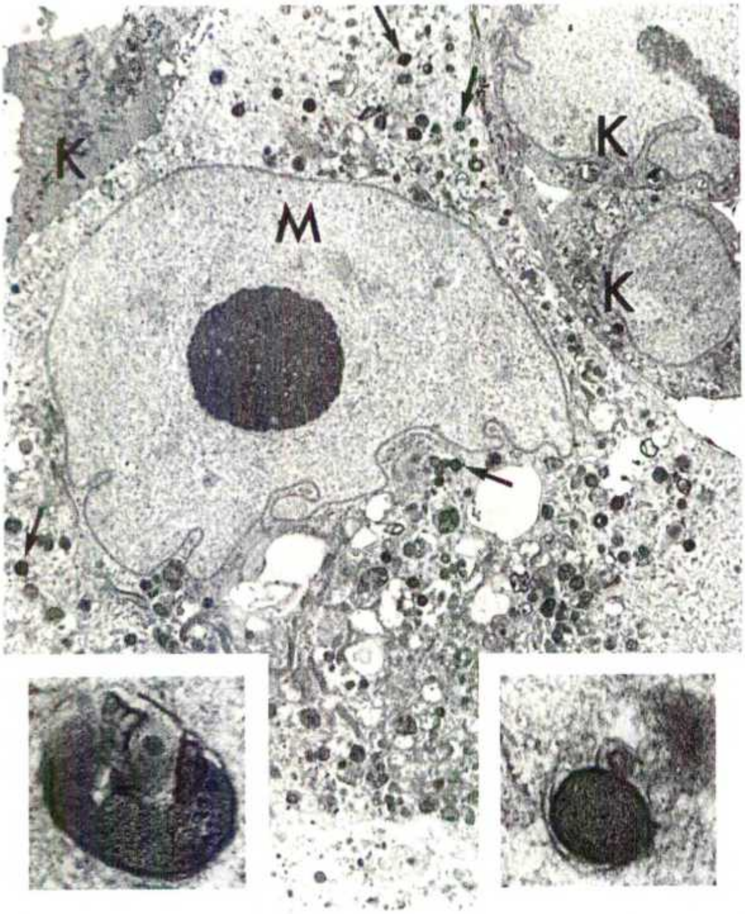

FIGURE 5. Electron micrograph of the melanocyte seen in Figure 4 (original × 7200). The melanocyte (**M**) is distinguishable from surrounding keratinocytes (**K**) by its clear cytoplasm which contains characteristic melanized granules (→), as seen in the two insets (original × 46,420). Tonofilaments and desmosomes are absent.

stimulate melanocyte growth using UVB, or psoralens and UVA which are known to induce melanocyte proliferation *in vivo*.[5,6,7,17-19] Recently, the direct stimulating effect of UVB exposure on melanogenesis and on the proliferation of cultured normal human melanocytes isolated from the epidermis has been described.[20-23] In such culture systems, melanogenesis was always stimulated by UVB whereas melanocyte growth was found to be stimulated in some studies and inhibited in others.

Under our experimental conditions, the stimulated melanocytes did not migrate in clusters, but remained separated from each other mimicking the epidermal melanin unit observed *in vivo*.

The significance of round dopa-positive cells in the epidermis is unknown. They may represent either inactive or decaying melanocytes.[24] We have no explanation for the higher density of round dopa-positive cells found in specimens treated with 8-MOP alone than in those treated with Hank's medium alone or UVA alone. However, the possibility cannot be excluded that, in spite of the fact that the 8-MOP cultures were kept in the dark, the low level of unfiltered light necessary for the experimental procedure was sufficient to stimulate the growth of melanocytes.

In our system, the fact that the melanocytes were cultured in the presence of keratinocytes and on fibroblasts in a collagen matrix may be relevant to their outgrowth under stimulation. Melanocyte growth-promoting activity has been found in an extract from a cultured foreskin fibroblast cell line[25] and stimulation of melanocyte growth by lethally irradiated fibroblasts *in vitro* has been observed.[26]

Under our experimental conditions, the melanocyte response to specific stimulation by UV light seems to be related to donor age. Only melanocytes obtained from donors less than 2 years of age were able to grow in the skin equivalent. This confirms previous observations made in other culture systems.[27] In our model, it will be of interest to determine if the outgrowth of older melanocytes can be induced under stimulation with UV light by the addition of hypothalamic bovine extract, known to contain melanocyte growth factor.[11]

Thus, the skin equivalent culture system allows a quantitative evaluation of the stimulation of melanocyte outgrowth by UVB or 8-MOP+UVA. This method should simplify basic investigations of melanocyte function such as the role of keratinocytes in the induction of melanocyte activity and the respective importance of melanocyte migration, activation and proliferation in response to various stimuli.

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Comparison of peanut agglutinin and S100 stains in the paraffin tissue diagnosis of Langerhans cell histiocytosis

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SUMMARY

We compared peanut agglutinin and S100 stains on paraffin embedded material from a variety of sites involved in Langerhans cell histiocytosis (LCH). The two techniques were comparable in terms of ease of performance, time taken and cost. Peanut agglutinin produced dense cell surface and paranuclear staining of the characteristic LCH cells seen in lesions, which was easier to distinguish than the more diffuse cytoplasmic staining produced by the S100 technique. This characteristic staining pattern is more specific than that for the S100 protein which is present in a variety of different cells, including some malignant histiocytes. We recommend that peanut agglutinin be used as a routine diagnostic test for all samples suspected of being LCH.

In Langerhans cell histiocytosis (LCH) the characteristic cell seen in the lesions is closely related to the Langerhans cell.[1] Both LCH cells and Langerhans cells have a characteristic tennis racquet shaped granule,[2,3] present in their cytoplasm. Both cells express the human thymocyte antigen CD1 on their cell surfaces [4,5] and both have intense α-mannosidase activity.[6] The S100 protein is also present in the cytoplasm of both cells.[7,8] When looking for these characteristic features to make or exclude the diagnosis of LCH there are, however, technical problems. Langerhans cell granules can only be seen on electron microscopy of suitably fixed material, and α-mannosidase and anti CD1 (OKT6) antibody stains require snap-frozen material. The only one of these stains that can be performed on routine paraffin-embedded material is the S100 stain.

S100 protein is not exclusive to Langerhans and LCH cells. It is present in melanocytes, glial tissue and a variety of other normal and tumour cells.[9] It has also been reported to be present in some cases of malignant histiocytosis.[8]

Peanut agglutinin, which is a lectin, has been reported to produce dense cell surface and paranuclear staining of LCH cells.[10] Peanut agglutinin produces diffuse staining of a large number of cells including keratinocytes, erythrocytes, macrophages,[11] and some lymphoid

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precursors.[12] However, the only other cells reported to produce the same characteristic binding as LCH cells are interdigitating reticulum cells of the lymph node and Reed-Sternberg cells in Hodgkin's lymphoma.[13,14]

We have examined material from a variety of sites (skin, lung, lymph node and spleen) involved in LCH and compared S100 and peanut agglutinin stains. We also applied both these stains in a variety of other diseases involving these organs.

METHODS

Tissue samples

Formalin fixed and paraffin embedded material was available from five skin biopsies, two lymph node, two spleen and one lung biopsy, all from different patients with histologically proven LCH involvement of the respective organs. For comparison, sections were also included from a benign melanocytic naevus and a juvenile xanthogranuloma of the skin, T cell lymphoma and Hodgkin's lymphoma of lymph nodes, B cell lymphoma involving the spleen, infection associated haemophagocytic syndrome involving the liver, and sections of liver and spleen from a patient with malignant histiocytosis. Sections from normal organs were also stained.

Sections 5 μm thick were cut, placed on glass slides coated with poly-L-lysine and then air-dried at 37°C. Sections from each specimen were stained with S100 or peanut agglutinin stains and the results compared.

Materials

Peanut agglutinin was obtained from Sigma Chemical Company. Rabbit anti-peanut agglutinin antibody, rabbit anti-cow S100 antibody and peroxidase-conjugated rabbit anti-mouse antibody were obtained from Dakopatts, Denmark.

S100-stain

The sections were dewaxed by immersing the slides in xylene for 3 min, and washed in alcohol and then in tap water for 5 min. The sections were then incubated for 15 min in protease 0·01% in phosphate buffered saline (PBS). Endogenous peroxidase was blocked by incubating with 1% hydrogen peroxide in methanol for 20 min followed by a wash in tap water and then PBS. The sides were blotted dry around the section, and then the sections covered with peroxidase-conjugated rabbit anti-cow S100 antibody at a dilution of 1:75 in PBS with 0·5% bovine serum albumin. This was left for 45 min then rinsed off with PBS. Peroxidase activity was then revealed using the Graham Karnovsky medium—diaminobenzidine 2 mg/10 ml of Tris buffered saline (TBS) pH 7·4 with 100 μl of 28% hydrogen peroxide added just before use—and incubating in the dark for 5 min.

Sections were then counterstained with haematoxylin, dehydrated in alcohol, cleared in xylene and mounted in DPX. Sections from a benign melanocytic naevus, where the S100 stained the melanocytes, were used as a positive control.

Peanut agglutinin stain

The sections were dewaxed by immersing the slides in xylene for 3 min, and washed in alcohol and then in tap water for 5 min. Endogenous peroxidase was blocked by incubating with 1% hydrogen peroxide in methanol for 20 min followed by a wash in tap water and then TBS pH 7·6.

The slides were blotted dry around the section and then the section covered with peanut agglutinin 10 mg/ml in TBS and incubated for 30 min. Following a wash in TBS the sections were incubated with rabbit anti-peanut agglutinin antibody at a dilution of 1 : 100 in TBS for 30 min and washed in TBS.

Sections were subsequently incubated with peroxidase-conjugated swine anti-rabbit antibody at a dilution of 1 : 50 in TBS for 30 min and then washed in TBS. Peroxidase activity was then revealed using the Graham Karnovsky stain as before. Sections were finally counterstained with haematoxylin, dehydrated in alcohol, cleared in xylene and then mounted in DPX.

Sections of kidney, where the peanut agglutinin stains erythrocytes and the cells of the distal convoluted tubule[11] were used as a positive control. A specificity control was carried out as

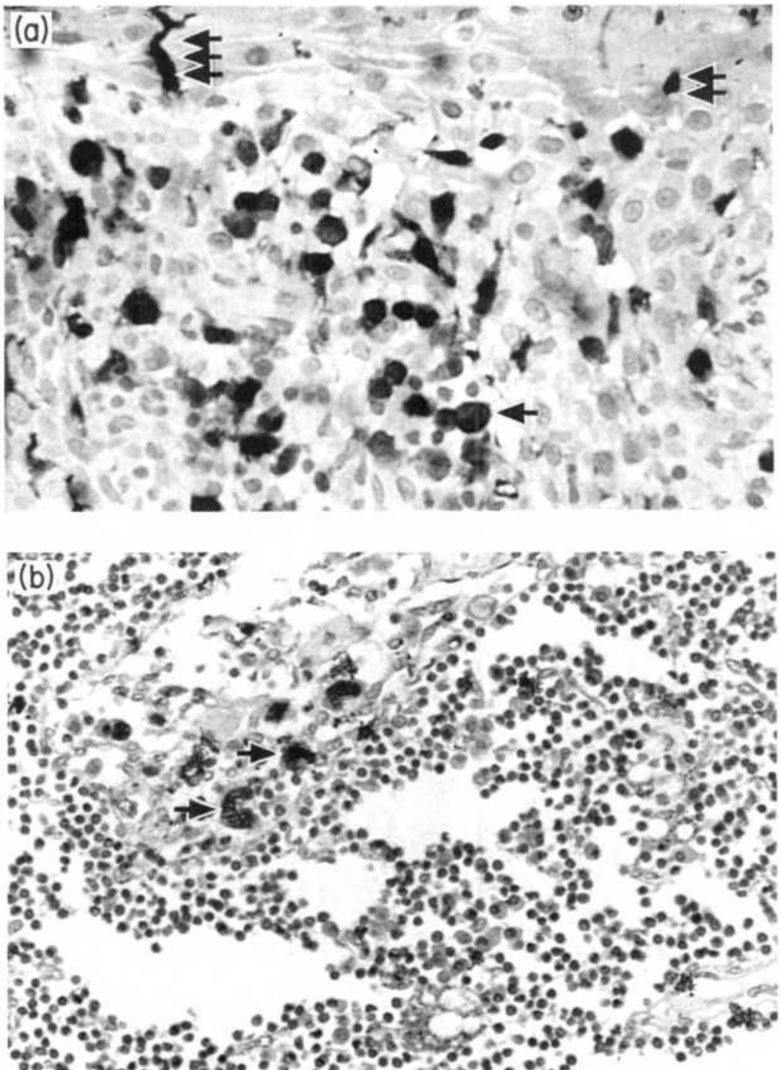

FIGURE 1. (a) Skin with LCH involvement stained for S100 protein. Single arrow indicates LCH cell, double arrow indicates melanocyte, triple arrow indicates Langerhans cell. (Original × 200). (b) Lymph node with LCH involvement stained for S100 protein. Arrows indicate, LCH cells. (Original × 800).

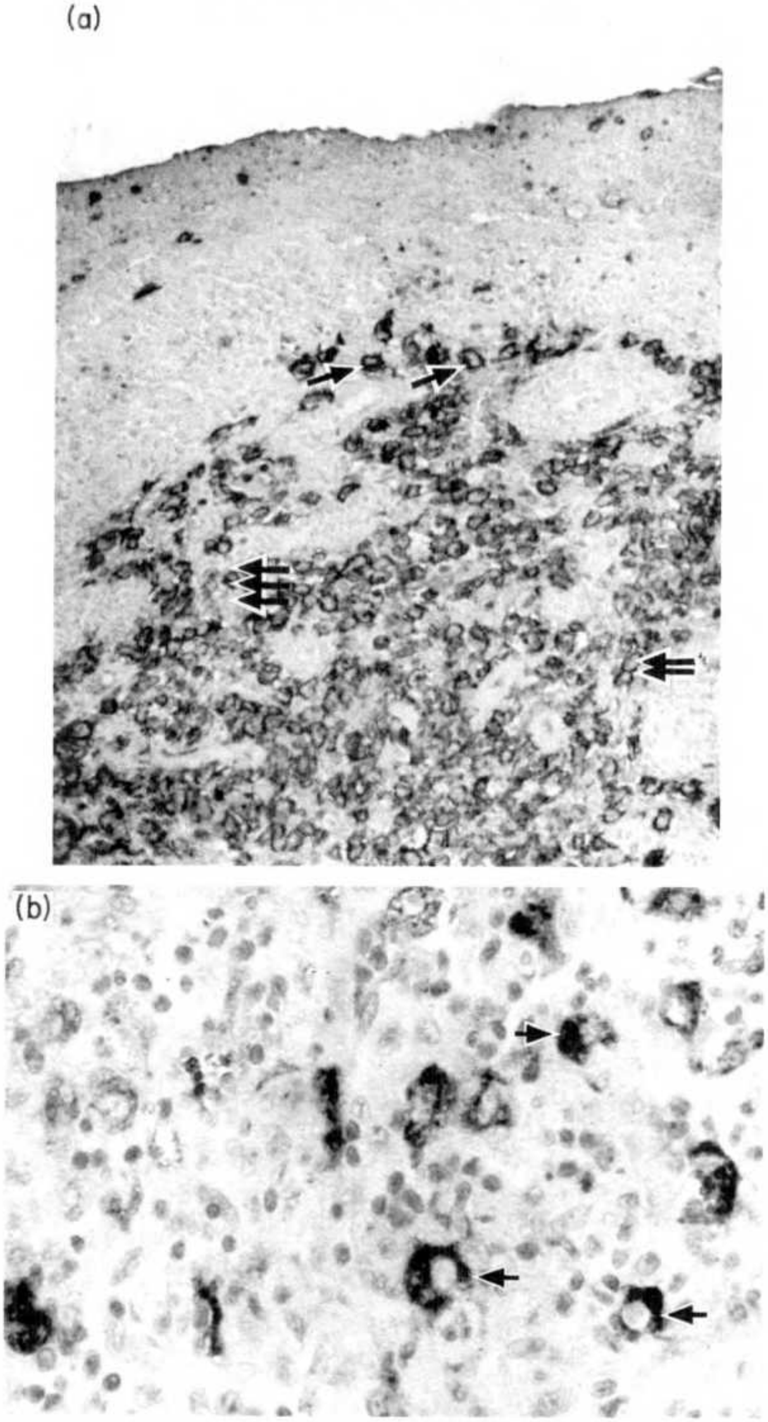

FIGURE 2. (a) Skin with LCH involvement stained with PNA. Single arrows indicate characteristic cell surface and paranuclear staining pattern of LCH cells, double arrow indicates cell surface staining alone and triple arrow indicates perinuclear staining alone. (Original × 100). (b) Skin with LCH involvement stained with PNA. Arrows indicate LCH cells showing perinuclear staining. (Original × 800).

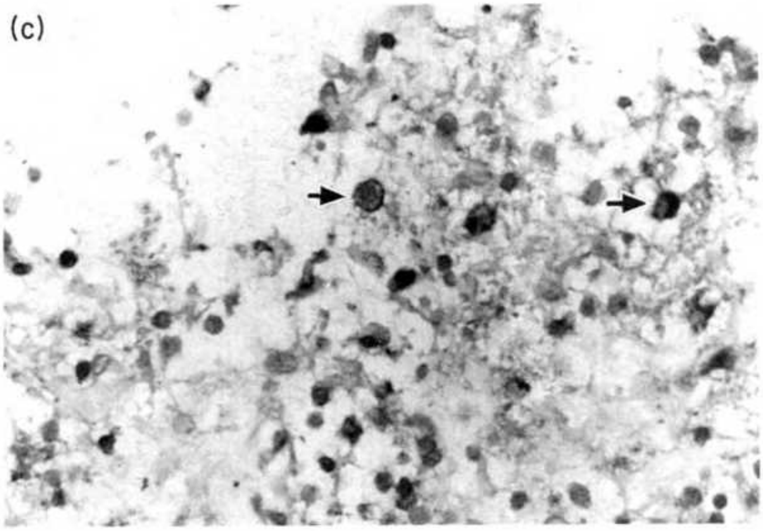

FIGURE 2. (c) Lymph node with LCH involvement stained with PNA. Arrows indicate LCH cells. (Original × 800).

above but incubating the lectin in the presence of 0·1 M D-galactose, to which sugar the lectin should preferentially bind.[11]

RESULTS

The S100 stain produced uniform cytoplasmic staining of the LCH cells in every section of skin, lung, spleen and lymph node involved in LCH. S100 was also present in melanocytes and in Langerhans cells in the skin (Fig. 1a) and interdigitating reticulum cells in lymph node (Fig. 1b).

Peanut agglutinin produced dense paranuclear and cell surface staining of the LCH cells in every section of skin (Figs 2a, b), lung, spleen and lymph node (Fig. 2c) involved in LCH. This pattern, although invariably present in each section was seen in only about 20% of the histiocytic cells. Other LCH cells showed cell surface staining alone or perinuclear staining alone. Reed-Sternberg cells in lymph nodes involved in Hodgkin's lymphoma, and interdigitating reticulum cells in normal and diseased lymph nodes produced the same dense cell surface and paranuclear staining. A diffuse staining of erythrocytes and keratinocytes occurred and melanocytes and Langerhans cells could not be distinguished.

Cells in the sections from juvenile xanthogranuloma, T and B cell lymphomas, infection associated haemaphagocytic syndrome and malignant histiocytosis were otherwise negative with both S100 and peanut agglutinin stains.

DISCUSSION

LCH is an uncommon disease with about 30–40 cases per year in the U.K.[15] It may present in a wide variety of ways to a number of different specialists, for example with recurrent pneumothoraces to a chest physician, with a 'pathological fracture' to an orthopaedic surgeon or with a skin rash to a dermatologist. It is often initially misdiagnosed, for example the skin lesions may be mistaken for seborrhoeic eczema. The diagnosis of LCH is important both for the management of the patient and in the prognosis it confers. It is, therefore, also an important disease to exclude.

Any special test should be sensitive, specific and easy to perform by a laboratory that does not often have to do it. It is preferable that it should be able to be performed on routine formalin-fixed paraffin embedded material, as the diagnosis can then be confirmed in retrospect or in cases that were not clinically suspected.

The characteristic LCH cell seen in the lesions has a number of diagnostic features. Birbeck granule-like structures are seen on electron microscopy,[3] and the cells are ATPase,[16] non-specific esterase,[17] α-D-mannosidase[6] and S100[8] positive. The cells also express CD4,[18] CD1[5] and HLA-DR[17] antigens.

Non-specific esterase is present in cells of myeloid or monocytic lineage and surface ATPase is a marker for histiocytic cells. α-D-mannosidase is present in many cells but intense positivity has been seen only in Langerhans and LCH cells. The CD4 antigen is present on helper T cells, thymocytes, monocytes, macrophages and some Langerhans cells, and CD1 is expressed on thymocytes and Langerhans cells. The Ia antigen occurs on dendritic cells including Langerhans cells, macrophages, monocytes and some activated T cells.

However, at present the only stain in routine use that can be performed on paraffin embedded tissue is the S100 stain. The S100 protein is not exclusive to LCH cells. It is present in some neurones, glial and Schwann cells of the nervous system,[19] interdigitating reticulum cells of the lymph node[20] and chondrocytes.[21] It is also present in melanocytes and normal Langerhans cells and this latter may cause confusion as to whether a collection of S100 positive cells is a normal reactive process or an abnormal collection of LCH cells. S100 protein has also been demonstrated in myoepithelial cells of sweat glands, salivary glands and breast, serous glands of the lung and sustentacular cells of the adrenal medulla. Among neoplasms, S100 protein has been reported in neurogenic tumours,[22] melanomas, sweat and salivary gland tumours, bronchoalveolar carcinomas, phaeochromocytomas and tumours of cartilage.[21] S100 has also been reported to be positive in some cases of malignant histiocytosis.[8]

Peanut agglutinin is a lectin. These are sugar-binding proteins or glycoproteins derived mainly from plants, but also from animals. Peanut agglutin stains a wide variety of cells including macrophages, erythrocytes and the distal collecting tubule cells of the kidney.[11] It binds to the carbohydrate of the cell membrane to produce a diffuse staining of the cell. However, the distinctive very dense cell surface and paranuclear staining of the LCH cells has additionally been described only in Reed-Sternberg cells in Hodgkin's lymphoma and interdigitating reticulum cells of the lymph node.[13,14] It does not stain malignant histiocytes or monoblasts in this characteristic pattern.[14]

We used a two step antibody technique as the one step technique, with peroxidase conjugated peanut agglutinin, has been found by some workers to be less reliable and less sensitive.[23] However, we have tried the one step technique with a commercial conjugate (Dako) at a concentration of 1:20 on several of the skin sections involved in LCH and obtained identical results to those obtained by the two step technique.

Both the S100 and peanut agglutinin stains identified LCH cells in skin, spleen, lymph node and lung. The techniques for each stain are comparable in terms of time, cost and simplicity of performance. The S100 protein is present in a large number of cells including some cases of malignant histiocytosis. The dense cell surface and paranuclear staining of LCH cells by peanut agglutinin, however, is easily recognized and is more specific.

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Adverse cutaneous drug reactions in AIDS

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SUMMARY

A maculopapular rash is reported in a girl with AIDS and *Pneumocystis carinii* pneumonia treated with co-trimoxazole. AIDS patients seem to be at increased risk of adverse drug reactions.

AIDS patients suffer from severe opportunistic infections which have to be treated by multiple drugs over long periods. Although the clinical manifestations of drug eruptions are extremely diverse in nature, it is important to recognize these, as some of the complications can be serious. It is often not possible to specify the responsible drug or the pathogenetic mechanism on the basis of the clinical appearance alone because the skin responds to a wide variety of stimuli, for instance viral infections, with a limited range of morphological reaction patterns.

CASE REPORT

A 6-month-old girl from Rwanda was admitted because of interstitial pneumonia, chronic diarrhoea and persistent high fever.

Laboratory studies showed an inflammatory syndrome (erythrocyte sedimentation rate, 100 mm/h) and hypergammaglobulinemia (IgG and IgE), positive HIV serology and HIV cultures, and a pattern of acquired immunodeficiency: OKT_4 8%, OKT_8 55%, diminished response of T cells to phytohaemagglutinin and concanavalin A, low production of alpha interferon and very low production of gamma interferon.

The child developed oesophageal candidosis, enteritis, purulent pneumococcal otitis and urinary *Klebsiella pneumoniae* infection.

After 3 weeks of treatment with co-trimoxazole she developed a widespread pruritic maculopapular rash, splenomegaly and neutropenia. The lesions disappeared when treatment was discontinued.

Skin biopsy showed oedema of the papillary dermis with a perivascular inflammatory infiltrate of monocytes and some polymorphonuclear leukocytes. Direct immunofluorescence was negative. A lymphocyte transformation test with co-trimoxazole was positive.

We concluded that this rash was an adverse cutaneous drug reaction.

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DISCUSSION

Mitsuyasu *et al.*[1] reported an increased incidence of cutaneous drug reactions (69%) associated with the administration of trimethoprim-sulphamethoxazole in patients with AIDS, with or without Kaposi's sarcoma. These patients did not have previously reported drug allergies and were not atopic. In all patients the rash resolved rapidly when treatment was discontinued.

Arndt *et al.*[2] estimated that the overall prevalence of drug-induced allergic skin reactions of 2% in the general population is an overestimate and that the real frequency of drug hypersensitivity is of the order of 3 per 1000. The only exceptions are the beta-lactams (penicillin G and ampicillin), co-trimoxazole, corticotrophin, erythromycin and transfusions which cause cutaneous reactions in more than 2% of cases. Cutaneous reactions to co-trimoxazole have been observed in 2·6–8% of patients.[3] The incidence of these complications in AIDS patients reported by Mitsuyasu *et al.*[1] is almost 10-fold higher. Other authors have also reported an increase in adverse reactions to trimethoprim-sulphamethoxazole in patients with AIDS.[4–6]

Allergic skin reactions may be considered as the result of a combination of predisposing host factors and facilitating environmental factors.

Considering the immunopathological mechanisms, skin allergies can be antibody mediated (type I, II and III of Gell and Coombs[7]), or cell-mediated (type IV). In practice however, these reactions do not necessarily occur separately.

The urticarial papules and the eczematous cutaneous lesions that we have observed in AIDS patients, can be attributed to the mediators of the type I reaction, characterized by the production of antibodies (mainly IgE) in response to sensitization by the allergen. Production of antibodies depends exclusively on B lymphocytes. Secretion of antibodies is modulated by complex regulatory mechanisms involving different subpopulations of T cells and macrophages. This regulation results from the equilibrium between helper and suppressor activity of T cells.

Some immune deficiency states, such as atopic dermatitis, can be accompanied by an increase of IgE together with a reduction in T suppressor cells.[8] In some AIDS patients we have found a raised level of IgE; however, regular measurements are needed to relate immunoglobulin levels to the degree of immune deficiency.

Farthing *et al.*[9] have reported an immune complex vasculitis with purpura in some patients with AIDS. Skin biopsy showed a characteristic leukocytoclastic pattern with deposition of complement in the vessels. Indirect immunofluorescence was positive using serum with a high titre of HIV, suggesting that the virus was the source of the antigen in this immune complex vasculitis. On the other hand, there are numerous other antigens, such as drugs, which can cause a type III reaction.

Recently Revuz[10] has reported the occurrence of toxic epidermal necrolysis, a severe drug reaction, in three patients with AIDS treated with trimethoprim-sulphamethoxazole for *P. carinii* pneumonia.

The mechanisms responsible for the epidermal necrolysis in this syndrome remain controversial. It has been suggested that an immunologically mediated cytotoxic reaction could explain the cutaneous changes as well as some of the haematological disturbances (anaemia and lymphopenia).[11]

Roujeau *et al.*[12] reported a reduction in the OKT_4/OKT_8 ratio with a decrease of T helper cells (OKT_4) in toxic epidermal necrolysis.

Thus, cutaneous drug reactions are complications which occur more frequently in patients with AIDS; however, their frequency and histopathological mechanism require further study in relation to the degree of immune deficiency.

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Assessment of the duration of action of terfenadine on histamine induced weals

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SUMMARY

In this study we aimed to quantify the reduction in area, perimeter and thickness of histamine induced weals 12, 18 and 24 h after a single oral dose of 120 mg terfenadine. Ten healthy volunteers were given 120 mg of terfenadine or placebo in a double-blind randomized two-period cross-over study. Twenty micrograms of histamine were then injected intradermally at 12, 18, and 24 h. The thickness of the resulting weal was measured by an A-scan pulsed ultrasound device. The area and perimeter of the resulting weal and flare were measured by tracing onto acetate sheets and using a digitizing tablet linked to a microcomputer. Just before each dose of histamine was injected, blood was taken to measure the level of the major metabolite of terfenadine. There was a significant difference between terfenadine 120 mg and placebo in the area and perimeter of the weal and the area and perimeter of the weal and flare at 12, 18 and 24 h, but no significant difference in weal thickness. Thus, terfenadine suppresses the wealing response caused by intradermally injected histamine over a 24 h period. It may be possible, therefore, to use a once daily dose of terfenadine.

Terfenadine is a selective H_1 receptor antagonist lacking significant penetration into the central nervous system. A standard method of studying H_1 receptor antagonists in man is to evaluate their ability to inhibit the skin reaction to intradermally injected histamine. In man, terfenadine is rapidly absorbed following a single oral dose. Peak terfenadine plasma concentrations are reached 1–2 h after administration. The ability of terfenadine to inhibit the skin reaction to intradermally injected histamine becomes evident within 24 h. Using a single dose of 20, 60 or 200 mg terfenadine, a dose dependent decrease in histamine weal area is produced reaching a maximum 3–6 h after administration. With the 60 and 200 mg doses significant reduction of weal area persists for 12 h, but with a dose of 40 mg there is no significant difference in histamine weal size from that produced by placebo at 12 h (Merrell Dow Pharmaceuticals, unpublished data on file). A multiple dose study of 20, 40 or 60 mg every 8 h for six doses, or 60 mg every 12 h for six doses revealed a statistically significant reduction in histamine weal size 8 h after five

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repeated doses.[1] The aim of the present study was to quantify the reduction in weal area, perimeter and thickness 12, 18, and 24 h after a single dose of 120 mg terfenadine using objective techniques.

METHODS

Ten normal subjects, five males and five females, aged 20 to 47 years, mean age 32·2 years, were recruited. All gave signed witnessed informed consent to the study which was approved by the Joint Ethics Committee of the South Glamorgan Area Health Authority and carried out in accordance with the provisions of the Declaration of Helsinki as amended in Venice (1983).

The study was conducted as a double-blind randomized two-period cross-over trial. Each subject received two treatments according to a predetermined randomization list prepared by Merrell Dow Research Institute: (1) Terfenadine 120 mg at 2000 h on the evening prior to commencing the study. (2) Placebo at 2000 h on the evening prior to commencing the study.

The terfenadine tablets were manufactured according to the commercial formulation (Triludan) and differed from the marketed product only in the embossed markings on the tablet surface. The placebo tablets were identical in formulation to the active tablets with the exception that terfenadine was replaced by lactose.

There was a washout period of 1 week between the end of one treatment period and the beginning of the next. Each subject attended the dermatology laboratory on the evening before the study began and was witnessed taking the study medication. Twelve hours after taking the tablets the subjects once again attended the dermatology laboratory. Blood was taken from all subjects in both parts of the study to measure serum 4-[1-hydroxy-4-[4-(hydroxydiphenyl methyl)-1-piperidinyl]butyl]-α, α-dimethylbenzene acetic acid levels, then 20 μg of histamine acid phosphate diluted in 0·1 ml of normal saline was injected intradermally using a 0·5 ml insulin syringe (monoject) into the flexor aspect of one forearm. The histamine was administered by the same investigator on each occasion. The injection was known to be in the correct position when a small bleb was raised in the skin as the histamine was introduced and disappeared within 3 min.

Immediately prior to each injection of histamine the thickness of the skin at the site of injection was measured using an A-scan pulsed ultrasound device[2] (Cutech Ltd.). The A-scan ultrasound sends a pulse of ultrasound into the body, and where it meets a boundary between tissues of different physical properties (such as dermis and fat), it is partially reflected, some of the energy being transmitted to the next boundary, and some being reflected as an echo. This echo is detected by a transducer and displayed on an oscilloscope which displays a trace of echo amplitude (A-scan) against time. The time between echoes is a function of the distance between the boundaries, and their height is related to the difference in physical properties of the tissues on either side of the boundaries.

Ten minutes after each injection of histamine the thickness of the weal was measured using the A-scan pulsed ultrasound device. The areas and perimeters of the weal and flare were also measured by tracing these lesions onto transparent acetate sheets. The areas were then computed using a digitizing tablet linked to a microcomputer.

Terfenadine undergoes rapid and extensive biotransformation on the first pass through the liver. It is 98% metabolized, the principal metabolite being 4-[1-hydroxy-4-[4-(hydroxydiphenyl methyl)-1-piperidinyl]butyl]-α, α-dimethylbenzene acetic acid (MDL 16455), which is the substance that was measured in the present study. These assays were performed at the Merrell Dow Research Institute. Plasma from the volunteers was added to an internal standard

(MDL 26042). MDL 16455 and the internal standard were then extracted with ethyl acetate and evaporated. The residue was reconstituted in mobile phase and chromatographed on a Spherisorb 5ODS2 HPLC column. The level of MDL 16455 in each sample was determined by comparison of the peak height ratios with those from a calibration graph.

The process of injection and measurement was repeated at 18 and 24 h after the study medication had been taken.

Statistical analysis

The data was analysed in the conventional manner for a two-period cross-over trial using *t*-tests on period differences and sums to estimate the treatment effect and any period and treatment interaction.[3]

RESULTS

Plasma levels of the principal terfenadine metabolite MDL 16455

MDL 16455 was not detected at any of the chosen time points when the subjects had taken placebo. MDL 16455 was detected at 12 h in all subjects given terfenadine. It was not detected in one subject at 18 h and in 3 subjects at 24 h.

Figure 1 shows the decrease in plasma concentration of the metabolite MDL 16455 with time following an oral dose of 120 mg of terfenadine.

Levels in the range 50–150 ng/ml of MDL 16455 were found at 12 h with a mean (± SEM) of 91·2 ± 13·0 ng/ml. In comparison, following a single oral dose of 60 mg of terfenadine using the identical assay system a mean value of 64·1 ng/ml has been detected.

At 18 h MDL 16455 levels ranged from undetectable to 69 ng/ml with a mean (± SEM) of 37·4 ± 7·1 ng/ml. After 24 h MDL 16455 levels ranged from undetectable to 44 ng/ml, mean (± SEM) 19·5 ± 4·6 ng/ml.

Area and perimeter of weal and flare

The data for both area and perimeter of the weal and flare was checked for consistency using the noncircularity index (NCI). The noncircularity index, a measure of the degree that a shape

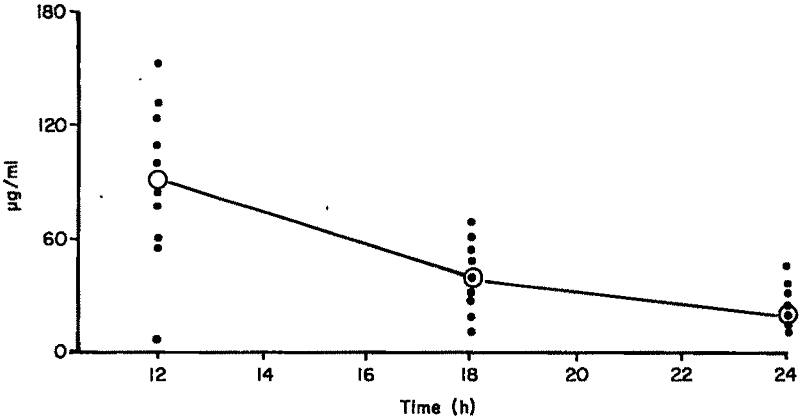

FIGURE 1. Plasma levels of MDL 16455 at 12, 18 and 24 h after a single oral dose of 120 mg of terfenadine ($n = 10$). MDL 16455 was not detected in one subject at 18 h and in 3 at 24 h. Points are individual values, open circles are means.

differs from a circle, is equal to the square of the perimeter divided by 4 π × area. The NCI is expected to be greater than or equal to 1 and the result for weal alone should be greater than for weal and flare combined. The data for all subjects in both groups of the study met these criteria. In order to correct for the extreme skewness of the distribution, a log transformation was used for both the area and perimeter.

Table 1 lists the geometric mean and the estimated 2·5 and 97·5 percentiles of the descriptive statistics of the area and perimeter of the weal alone at 12, 18 and 24 h. The geometric mean is calculated by transforming the mean of the log transformed values back to the original scale. The estimated 2·5 and 97·5 percentiles are obtained from antilogs of the mean ± 1·96 standard deviation values for each variable.

Using *t*-tests to estimate the effects of terfenadine on histamine induced weals revealed statistically significant differences from placebo at the three chosen time points for both area and perimeter of the weal alone. Table 2 lists the *P* values, mean differences and 95% confidence intervals.

T-tests were used to estimate whether there was any interaction between the two periods and the treatment. The effectiveness of terfenadine was no different in the two groups (used first in one and second in the other) and there was no evidence of a carry-over effect from one period to the other.

An attempt was made to ascertain whether there was any correlation between weal area and plasma levels of MDL 16455 at 12, 18 or 24 h. The correlation between log weal area and plasma level of MDL 16455 was not significant at any of the three time points (sample correlation: −0·41 at 12 h, −0·62 at 18 h and −0·27 at 24 h), but was negative in the expected direction.

Table 3 lists the geometric mean and the estimated 2·5 and 97·5 percentiles of the area and perimeter of the weal and flare at 12, 18 and 24 h.

TABLE 1. Geometric mean and estimated 2·5 and 97·5 percentiles for the area and perimeter of the weal alone following an intradermal challenge of 20 μg of histamine acid phosphate at 12, 18 and 24 h after a single oral dose of 120 mg of terfenadine or placebo ($n = 10$)

			Original scale	
Measurement	Time (h)	Treatment	Geometric mean	Estimated 2·5 and 97·5 percentiles
Area (mm^2)	12	Terfenadine	87·2	38·0–200·0
		Placebo	169·5	62·3–461·1
Perimeter (mm)	12	Terfenadine	37·2	24·5–56·4
		Placebo	52·6	29·4–94·0
Area (mm^2)	18	Terfenadine	124·1	36·0–427·5
		Placebo	191·5	88·8–412·9
Perimeter (mm)	18	Terfenadine	44·0	19·4–99·6
		Placebo	55·3	34·1–89·8
Area (mm^2)	24	Terfenadine	131·9	64·7–269·0
		Placebo	197·0	59·2–656·1
Perimeter (mm)	24	Terfenadine	44·2	30·8–63·4
		Placebo	58·3	29·1–116·7

TABLE 2. Effect of terfenadine on area and perimeter of weal alone following an intradermal challenge of 20 μg of histamine acid phosphate at 12, 18 and 24 h after a single oral dose of 120 mg of terfenadine or placebo ($n = 10$).

		Log transformed scale		Original scale		
Measurement	Time (h)	Mean difference (T−P)	95% confidence interval	Geometric mean difference (T−P)	95% confidence interval	*P*
Area	12	−0·29	−0·40 to −0·18	−48·6%	−60·1% to −33·7%	<0·01
Perimeter	12	−0·15	−0·22 to −0·08	−29·2%	−39·5% to −17·2%	<0·01
Area	18	−0·19	−0·33 to −0·05	−35·2%	−52·9% to −10·8%	<0·05
Perimeter	18	−0·10	−0·18 to −0·02	−20·5%	−34·4% to − 3·6%	<0·05
Area	24	−0·17	−0·30 to −0·05	−33·1%	−49·5% to −11·3%	<0·05
Perimeter	24	−0·12	−0·21 to −0·03	−24·2%	−38·0% to − 7·4%	<0·05

T = terfenadine, P = placebo

TABLE 3. Geometric mean and estimated 2·5 and 97·5 percentiles for the area and perimeter of the weal and flare following an intradermal challenge of 20 μg of histamine acid phosphate at 12, 18 and 24 h after a single oral dose of 120 mg of terfenadine or placebo ($n = 10$)

			Original scale	
Measurement	Time (h)	Treatment	Geometric mean	Estimated 2·5 and 97·5 percentiles
Area (mm^2)	12	Terfenadine	862	252–2951
		Placebo	2331	424–12804
Perimeter (mm)	12	Terfenadine	123	60–252
		Placebo	230	75–703
Area (mm^2)	18	Terfenadine	971	325–2906
		Placebo	1984	450–8740
Perimeter (mm)	18	Terfenadine	133	70–253
		Placebo	195	77–493
Area (mm^2)	24	Terfenadine	1296	458–3667
		Placebo	2243	755–6661
Perimeter (mm)	24	Terfenadine	148	79–277
		Placebo	206	97–438

T-tests showed a statistically significant difference between the effects of terfenadine and placebo on the area and perimeter of histamine induced weal and flare at each of the three time points. Table 4 lists the *P* values, mean differences and 95% confidence intervals.

T-tests were used to estimate whether there was any interaction between the two periods and the treatment. At 12 and 18 h there was no significant interaction, but at 24 h there appeared to be an interaction between treatment and periods ($P < 0{\cdot}05$ for area and perimeter). An analysis based just on period 1 data, the recommended procedure in such circumstances, revealed a statistically significant difference between treatments for both area and perimeter ($P < 0{\cdot}05$), with greater reductions on active treatment than those shown in Table 4; −68·3% and −49·9%, respectively.

TABLE 4. Effect of terfenadine on area and perimeter of weal and flare following an intradermal challenge of 20 μg of histamine acid phosphate at 12, 18 and 24 h after a single oral dose of 120 mg of terfenadine or placebo ($n = 10$)

		Log transformed scale		Original scale		
Measurement	Time (h)	Mean difference (T − P)	95% confidence interval	Geometric mean difference (T − P)	95% confidence interval	P
Area	12	−0·43	−0·59 to −0·28	−63·0%	−74·1% to −47·2%	<0·001
Perimeter	12	−0·27	−0·40 to −0·15	−46·6%	−60·2% to −28·4%	<0·01
Area	18	−0·31	−0·45 to −0·18	−51·0%	−64·2% to −33·1%	<0·01
Perimeter	18	−0·16	−0·30 to −0·03	−31·5%	−49·7% to −6·8%	<0·05
Area	24	−0·24	−0·39 to −0·09	−42·2%	−59·1% to −18·3%	<0·01
Perimeter	24	−0·14	−0·26 to −0·03	−28·3%	−45·5% to −5·7%	<0·05

T = terfenadine, P = placebo

Weal thickness

The mean thickness of the histamine induced weals (± SEM) at 12 h was 1·32 ± 0·14 mm for placebo and 1·20 ± 0·14 mm for terfenadine. At 18 h the values were 1·41 ± 0·05 mm for placebo and 1·64 ± 0·16 mm for terfenadine. The mean weal thickness at 24 h was 1·47 ± 0·07 mm following placebo and 1·83 ± 0·20 mm following terfenadine. No significant treatment or time effects were found for weal thickness. However, the data was not regarded as of optimal reliability because of a technical failure in the equipment.

DISCUSSION

In this study it was decided to evaluate the effects of terfenadine on histamine induced skin weals in man 12, 18 and 24 h after ingestion of a single oral dose because it is known that single doses of 60 mg and 200 mg produced a significant reduction in weal area lasting for at least 8 h.[1] Another study showed that 12 h after a dose of 40 mg the histamine induced weal size did not differ from that following placebo, but doses of 60 mg and 120 mg did produce significant reduction in weal size.

In the present study the effect of terfenadine, in reducing the size of the induced weal and flare, was sufficiently large and consistent to reach conventional levels of statistical significance using 10 subjects irrespective of whether weal alone or weal and flare was studied, whether area or perimeter was measured, and whether 12, 18 or 24 h had elapsed since the drug was taken. The consistency of the response in this respect strongly suggests that statistical significance was not merely the result of multiple significance testing. Quantitatively, the percentage inhibition decreased with time and was greater for weal and flare than for weal alone. This study has demonstrated, therefore, an inhibition, judged to be clinically important and attaining statistical significance at 12, 18 and 24 h after ingestion of a single oral dose of terfenadine 120 mg, of the weal and flare area and perimeter following an intradermal injection of 20 μg of histamine acid phosphate.

No significant change in weal thickness was demonstrated. However, in previous work, following five doses of terfenadine 60 mg over 2½ days, we have shown a significant reduction in weal thickness using the A-scan pulsed ultrasound device in histamine induced weals.[4]

It was not surprising that we did not find any correlation between weal area and the plasma levels of 4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-α, α-dimethylbenzene acetic acid (MDL 16455). There is a definite difference in timing between peak plasma concentration and maximal effect. Peak plasma concentrations of MDL 16455 following a single oral dose of terfenadine 60 mg are of the order of 300 ng/ml and are reached 1 to 2 h after administration of terfenadine. Following a single oral dose of terfenadine 20, 60 or 200 mg the maximum effect occurs by the 4th hour after dosing.[1] This delay is not clearly understood. In future studies, sampling of tissue MDL 16455 levels from suction blister fluid would permit assessment of whether this is a better reflection of the binding of terfenadine to H_1 receptors in the skin.

A circadian rhythm in the skin reaction to intradermal histamine has been demonstrated in man. The reaction appears to be minimal towards noon and maximal close to midnight. This may affect the effectiveness of an antihistamine depending on when the dose is taken. Sixty milligrams of terfenadine administered at 0700 h resulted in an increased time to maximal effect (9·7 h) and a longer duration of effect (23·0 h) compared with the same dose taken at 1900 h (4·1 h and 14·6 h, respectively). However, the degree of maximal inhibition was greater when the drug was administered at the latter time (−38·5% compared with −66·2%).[5] Although we did not have a 24 h profile of weal and flare areas the results we have for placebo could be interpreted as following a circadian rhythm. However, the results with 120 mg terfenadine appear to abolish the circadian response and we were still able to demonstrate that this single dose of terfenadine was effective for up to 24 h despite administering it in the evening when a shorter duration of effect would be expected. We infer that it may well be possible to use a single daily dose of terfenadine.

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Geometrical considerations in the statistical analysis of histamine induced weal studies

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The accompanying paper[1] reports the results of a phase II crossover trial in volunteers, demonstrating that the action of terfenadine in inhibiting the histamine-induced weal and flare reaction in man persists for 24 h. The purpose of this note is to develop some two-dimensional geometrical characteristics representative of tracings of these lesions that will help investigators to check for consistency and produce more appropriate statistical analyses of data from such studies. These considerations should be taken into account as well as the more general (but insufficiently heeded) principles of correct choice of study design—in particular, whether or not a cross-over trial[2] is appropriate—and what constitutes an adequate number of subjects to yield a good prospect of clinically useful information.[3]

CHARACTERISTIC APPEARANCE AND CONSISTENCY CHECKS

Figure 1 shows a characteristic histamine reaction. Two regions are measured, the weal alone and the weal plus flare. Each region is measured by a process of planimetry, involving tracing onto an acetate sheet and digitising. Generally, as in Figure 1, the weal lies wholly inside the area characterized by the flare. One anomalous lesion was produced (Figure 2) in which the weal lay outside, but contiguous with, the flare.

Several measures are available to characterize the extent of the lesion produced. Of these, the area (A) and perimeter (P) turn out to be most readily measured and used. A useful consistency check involves calculating the noncircularity index $NCI = P^2/4\pi A$ for each lesion. This is a measure of the degree to which the shape differs from a circle. For a given perimeter, the shape that maximises the enclosed area is a circle, so we expect to find $NCI \geqslant 1$, with equality only for an exactly circular shape. This relationship assumes that the shape being measured is perfectly flat; departures of the lesion from flatness could lead to an NCI slightly under 1, but this could not happen with measures from a tracing. The only other way in which an NCI value lower than 1 could arise is from measurement error—imprecision could lead to values very slightly lower than 1, whereas values substantially less than 1 would indicate gross errors, necessitating repetition of the digitisation.

The property $NCI \geqslant 1$ holds also for peculiar shapes such as Figure 2; in fact the largest NCI values in the study arose from this lesion. A further useful consistency check involves comparing

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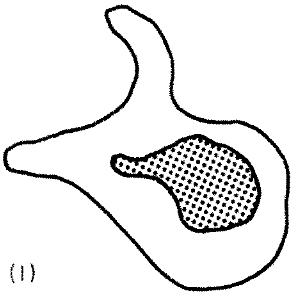

FIGURE 1. Diagram of a typical weal and flare. Shaded area indicates the weal.

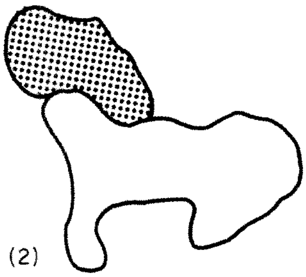

FIGURE 2. Diagram of an atypical weal and flare in which the weal (shaded area) fell outside but contiguous with the flare.

NCI for the weal with that for the weal plus flare; the latter is usually smaller, because of the characteristic 'fried egg' appearance (Fig. 1).

Differences in areas of lesions produced by different treatments are likely to be more striking than corresponding differences in perimeters; thus a 20% reduction in perimeter would correspond approximately to a 36% reduction in area, assuming NCI values were similar for the two groups of lesions.

CHOICE OF TRANSFORMATION

The data analysed did not conform to a normal distribution, and this is probably typical. Nevertheless, as with most data sets comprising measurements, parametric methods of analysis are preferable to non-parametric ones, in that they yield readily interpreted quantitative estimates. In this case, a suitable transformation of scale can produce an acceptably normal distribution. When areas and perimeters are being measured, it seems appropriate to use compatible scalings for the two measurements. The two possibilities are: (i) P untransformed, A square-root transformed, (ii) P and A both log-transformed.

The former is equivalent to assuming that linear dimensions conform to the normal distribution. The latter corresponds to the assumption of log normal distributions, i.e. that both log A and log P follow normal distributions. In either case, quantitative estimates of treatment effects on the transformed scales should be similar for both area and perimeter measures.

CONCLUSION

The analysis of weal and flare planimetry data is made more satisfactory by using the consistency checks that arise from the geometric properties of the lesion and by choosing transformations of scale for areas and perimeters that preserve their inter-relationship.

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Relief of experimentally induced pruritus with a novel eutectic mixture of local anaesthetic agents

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SUMMARY

The effectiveness of a eutectic mixture of the local anaesthetics lignocaine and prilocaine (EMLA® cream) in relieving artificially induced pruritus was examined in 20 volunteers. The study was conducted in two parts. In part one, the effect of EMLA on the threshold concentration of histamine necessary to induce pruritus was assessed by a double-blind placebo controlled method. In part two, the effect of EMLA on the perception of pruritus induced by the artificial pruritogens cowhage and papain was assessed by a single-blind method.

Some subjects were less sensitive to histamine after placebo cream treatment, but all subjects showed a marked reduction in sensitivity to histamine after EMLA treatment. The difference between EMLA and the placebo treatment was statistically significant.

The EMLA preparation was found to be effective in alleviating pruritus induced by cowhage and papain and this effect was also statistically significant.

Thus, EMLA cream was found to be effective in reducing experimentally induced pruritus and, therefore, may be useful in some clinical circumstances where persistent itch is a distressing symptom.

Pruritus is common in dermatological disease, but there are few ways of alleviating this symptom other than by removing the disorder causing it. As this ideal may not be met in practice, effective treatments directed towards the symptom of itch are needed. The sensation of itch appears to be mediated by the same neural pathways as those responsible for pain. For this reason there is pharmacological logic in attempting to improve pruritus by the use of locally administered anaesthetic agents. Unfortunately, absorption through intact skin of the routinely formulated 'caine' type anaesthetic preparations is insufficient to give any practical benefit, although they may be more effective when applied to mucosal surfaces.[1] The eutectic mixture of the bases of lignocaine and prilocaine is an oil at room temperature, and when mixed with water

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into an emulsion has properties such that the local anaesthetic bases can penetrate intact skin in amounts sufficient to induce surface anaesthesia. The local anaesthetic properties of this Eutectic Mixture of Local Anaesthetics (EMLA®, Astra Pharmaceuticals Ltd.) has been extensively documented.[2-9] To date, there have been no reports on the effectiveness of EMLA in relieving pruritus.

The aim of the present study was to compare the effects of EMLA and a placebo cream on the perception of pruritus produced by two experimental methods. The first method involved measuring the threshold concentration of histamine necessary to induce pruritus before and after treatment with EMLA or placebo cream. The second method examined the effect of EMLA on pruritus induced by the artifical pruritogens cowhage and papain.

METHODS

Twenty normal subjects, (five males, 15 females), aged between 19 and 50 years (median 30 years), who had given their written informed consent, took part in the study. The subjects had normal skin and none had a history of skin disease. None of the subjects was taking any medication at the time of the study.

Determination of itch threshold

The histamine induced itch threshold was determined for each subject. Test solutions of histamine (as histamine acid phosphate, diluted in sterile water) were prepared in increasing concentrations, as shown in Table 1. An area of 1·5 × 15 cm was delineated on the volar aspect of the forearm, (site 1) and 100 μl of the most dilute test solution was applied to a 1 cm^2 area within this region. The skin was scarified using a hypodermic needle so that the stratum corneum was disrupted and passage of the test material into the viable cell layers permitted. After 2 min the subject was asked to report whether there was any sensation of itching or stinging. If there was

TABLE 1. Subjects' responses to varying concentrations of histamine

	No. of subjects		
Histamine concentration (μmol)	Untreated control response (Site 1)	EMLA response (Sites 2 and 3)	Placebo response
0·013	0	0	0
0·025	0	0	0
0·051	4	0	0
0·102	9	1	4
0·214	3	4	7
0·407	2	7	5
0·815	1	4	2
1·09	1	2	0
1·63	0	0	1
3·26	0	2	1
Total:	20	20	20

no itching or stinging, the procedure was repeated at a new site on the delineated area with the next highest concentration of histamine. By repeating this procedure until itching or stinging occurred, a threshold concentration of histamine was obtained for each subject. This is the untreated control response shown in Table 1.

Effect of EMLA and placebo on itch threshold

Two further areas, 1·5 × 15 cm, were delineated, one on each forearm, (site 2 on the left arm and site 3 on the right arm). Placebo cream (in which the eutectic mixture of the local anaesthetic bases was substituted with Miglyol® 812 oil), was applied to one forearm and the EMLA cream was applied to the other test area. The placebo cream was visually and cosmetically identical to the test cream. The procedure was double-blind and the site of application, (i.e. right or left forearm), was randomized for each cream. Each site of application was occluded with plastic film for 120 min. After this time, the cream was removed, the sites inspected for any local adverse reactions and the histamine itch threshold determined for the EMLA and placebo treated test sites, using the same method and the same dilutions of histamine as described above.

Effect of EMLA on itching induced by artificial pruritogens

This was an open evaluation of the anti-pruritic effect of EMLA, carried out in the same subjects as in the first part of the study. A fourth site on the forearm, 1·5 × 15 cm was delineated. Ten subjects were randomly allocated to receive cowhage and 10 subjects papain. The test substances were 0·1 ml of a solution containing 50 μg/ml of papain (Sigma Chemical Company), or 1 mg of cowhage (W.Stevens, Royal Arcade, Cardiff). The test substances were applied to an untreated area of the skin and were sufficient to cause itching or stinging. The same concentration of pruritogen was then applied to a 1 cm^2 area which had previously been treated with EMLA for 120 min under the same conditions as described above. Although this test was performed open with regard to the investigator, the subjects were not told that the only cream applied was EMLA, but were informed that they were to receive either EMLA or the placebo cream.

Subjects were asked to report the degree of itching on untreated skin using a 100 mm visual analogue scale (VAS) with a range from 'not noticeable' to 'very irritating or noticeable'. They were also asked to assess the itching on a 4 category verbal rating scale (VRS) as 'none', 'mild', 'moderate' or 'severe'. This assessment was repeated for the site pre-treated with EMLA cream. In addition, subjects were asked to make an explicit comparison between the degree of itching felt when the pruritogen was applied to the untreated site compared to that felt when applied to the EMLA cream treated site.

RESULTS

Threshold sensitivity to intracutaneous histamine

The threshold sensitivity of subjects to varying concentrations of histamine on the untreated site, (site 1), is shown in Table 1. The frequency distributions of the sensitivity of subjects to histamine on EMLA and placebo treated areas (sites 2 and 3), are also shown in Table 1. Following treatment with EMLA, all subjects were less sensitive to histamine; 14 of the 20 subjects required a solution 2 or 3 concentration increments stronger than on the untreated site to provoke a reaction. In contrast, with the placebo treatment, four subjects showed no loss of sensitivity and only six required a solution of more than 1 concentration increment stronger than that required to provoke a reaction on untreated skin. An EMLA versus placebo comparison was carried out using the Wilcoxon signed rank test applied to the change in sensitivity data. The decrease in sensitivity to histamine was significantly greater on the EMLA treated site than on the placebo treated site ($P < 0{\cdot}01$).

Sensitivity to cowhage and papain

All 10 subjects in each group experienced less itching or stinging on the EMLA treated site compared with the untreated site. The results of the VRS and VAS measures of skin irritation are shown in Tables 2 and 3. The Wilcoxon signed rank test was used for an 'untreated' versus 'treated' skin comparison for both the VRS and VAS data. The cowhage and papain groups were tested separately and all comparisons showed significantly greater irritation on the untreated area than on the treated area, (cowhage VRS: $P < 0{\cdot}01$; cowhage VAS: $P < 0{\cdot}01$; papain VRS: $P < 0{\cdot}05$; papain VAS: $P < 0{\cdot}01$). The mean VAS score for cowhage was rather greater than that for papain, and a similar difference for the VRS data was also apparent. Analysis of the data relating to histamine itch threshold for each individual showed that subjects in the papain group required stronger histamine solutions to provoke a reaction than was the case for subjects in the cowhage group. Thus differences in sensitivity to the pruritogens probably reflect no more than inter-subject variation.

Local adverse reactions

There were two cases each of oedema and redness noted on the EMLA treated sites and none noted on the placebo treated sites. Both cases were assessed as mild. As has been reported previously, EMLA produced a greater degree of blanching than the placebo cream. No other local reactions were observed, and all subjects completed the study.

TABLE 2. Sensitivity to applied pruritogens: verbal scale response

		No. of subjects	
Treatment	Patient's assessment	Cowhage	Papain
Untreated skin	Mild stinging/itching	0	6
	Moderate stinging/itching	8	4
	Severe stinging/itching	2	0
EMLA treated skin	No stinging/itching	8	6
	Mild stinging/itching	2	4

TABLE 3. Sensitivity to applied pruritogens: visual analogue scale response

	Visual analogue scale rating (mean ± SD)	
	Cowhage	Papain
Untreated skin	49·1 ± 25·31	29·6 ± 11·8
EMLA treated skin	5·1 ± 9·07	4·5 ± 5·78

DISCUSSION

This study has demonstrated that a topical anaesthetic preparation, EMLA, is effective in reducing pruritus induced by both histamine and the artificial pruritogens cowhage and papain. Interestingly, itching was not completely abolished in any of the subjects. Explanations for this incomplete effect include the possibility that for some reason the preparation had an incomplete anaesthetic effect, that the sensation of itch is not entirely mediated by the pathways blocked by local anaesthetic agents, or that there were inaccuracies in the threshold recording system used. Nonetheless, we believe that the striking decrease in pruritus recorded suggests that the preparation may still have important therapeutic applications. Our study was not designed to assess the duration of action of EMLA and clearly this aspect of its use needs further investigation.

Itch is probably the most frequently voiced complaint of those suffering from skin disease and is a symptom that is difficult to assist pharmacologically. Our therapeutic inadequacy is partially due to the lack of models in which to assess candidate drugs. Cowhage and papain were studied in addition to histamine as these exogenous enzymes cause severe itching. The itching produced by these agents is perceived by many subjects as being more similar to naturally occuring itch. Although no direct comparison with placebo cream was made in this part of the study, the results clearly indicate that EMLA cream was very effective in eliminating or reducing the itching sensation. Thus, although neither model of itch employed in the present study is ideal, the combination of the two probably represents the best available.

The anatomical and neurophysiological basis of itch is complex and has been well reviewed by Fjellner.[10] It is generally held that the sensation of itch is mediated by the neural pathways that mediate pain, although there is considerable doubt as to whether it is in fact a form of pain. It is well known that topical anaesthetics can relieve itch when preparations are applied to mucosal surfaces. Unfortunately, the ester type 'caine' agents employed for this purpose (e.g. cinchocaine, amethocaine) may act as sensitizers and all are poorly absorbed from skin outside mucosal sites. In contrast, the potential for sensitization by the active ingredients of EMLA (lignocaine and prilocaine) is very low[11] and these local anaesthetics are well absorbed from the eutectic mixture. The formulation of EMLA, in which lignocain and prilocaine crystals are mixed, produces an emulsion droplet which contains approximately 80% active local anaesthetic agent, in contrast to a conventional emulsion of lignocaine in which there is only 20% active agent present in each droplet. The clinical effect is therefore enhanced by the increased concentrations of the active agents at the cream–skin interface.

A disadvantage of EMLA is the time required for onset of action. One study which assessed the effect of EMLA on venepuncture pain showed that the minimum effective application time for the induction of cutaneous anaesthesia was 45 min.[4] It may, nonetheless, be useful in some clinical circumstances (for example, in lichen simplex chronicus) where persisting itch is a distressing symptom, by breaking the itch-scratch-itch cycle.

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Remarkable response of lipoid proteinosis to oral dimethyl sulphoxide

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SUMMARY

We report the successful treatment of lipoid proteinosis in a 41-year-old man using oral dimethyl sulphoxide. The initial dosage was 40 mg/kg/day, which was then increased progressively to 60 mg/kg/day. After 3 years of treatment, the patient's skin lesions, hoarseness of voice and abnormal oesophageal function improved remarkably. No side-effects were noted except for a garlic-like smell on the patient's breath.

Hyalinosis cutis et mucosae, also known as lipoid proteinosis, is a rare, autosomal recessive disorder characterized by widespread deposition of hyaline material in the skin, mucosae and various internal organs. The disorder runs a chronic and benign course. Although it causes functional and cosmetic problems, the prognosis of this disease is good and mortality is low except in cases of severe untreated laryngeal obstruction.

No effective treatment for lipoid proteinosis has been described to our knowledge. Hoarseness may be relieved temporarily by surgical removal of vocal cord infiltrates. Tracheostomy is necessary in some cases due to laryngeal obstruction.[1] Facial lesions may be treated by dermabrasion, chemical skin peeling and blepharoplasty.[2] We have previously reported a patient with this disease.[3]

We report here the results of 3 years treatment with oral dimethyl sulphoxide. The possible mechanism of the response of lipoid proteinosis to dimethyl sulphoxide is discussed.

CASE REPORT

A 41-year-old man presented with hoarseness and asymptomatic skin eruptions on the face, axillae, perineum and limbs which had been present since he was a teenager. Physical examination showed multiple small skin-coloured bead-like papules along the eyelid margins, discrete or grouped hyperkeratotic papules and plaques over the sides and knuckles of the fingers, extensor aspects of the forearms, elbows, axillae, shins and perineum. The mucous

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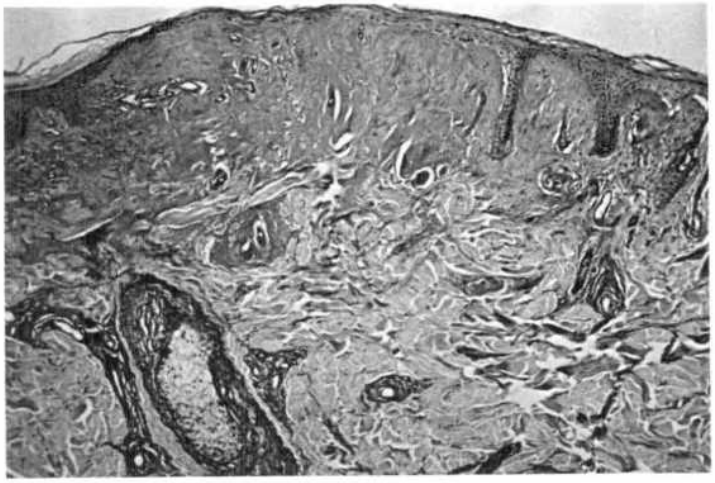

FIGURE 1. PAS-positive material deposited in the dermis and around blood vessels and skin appendages (original × 250).

membranes of the oral cavity and larynx, including the vocal cords, were irregular with yellowish-white patches.

Biopsies of affected skin and mucous membrane showed diffuse deposition of hyaline material in the dermis and around the blood vessels, sweat glands and hair follicles. The hyaline material reacted strongly with PAS (Fig. 1), alcian blue and oil red o stains. On electron microscopic examination, the hyaline material was composed of a mixture of an amorphous substance and ill-defined, wavy and stringy, fine filaments interspersed with frayed collagen fibres.

Laboratory studies, including haematology, urinalysis, fasting blood sugar, blood lipids, lipoproteins, blood urea nitrogen, serum creatinine, electrolytes, serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were all normal. Serum electrophoresis revealed persistently increased IgG.

Treatment

After the diagnosis of lipoid proteinosis, the patient was started on oral dimethyl sulphoxide, 40 mg/kg/day. After 2 months, the dosage was increased progressively and maintained at 60 mg/kg/day.

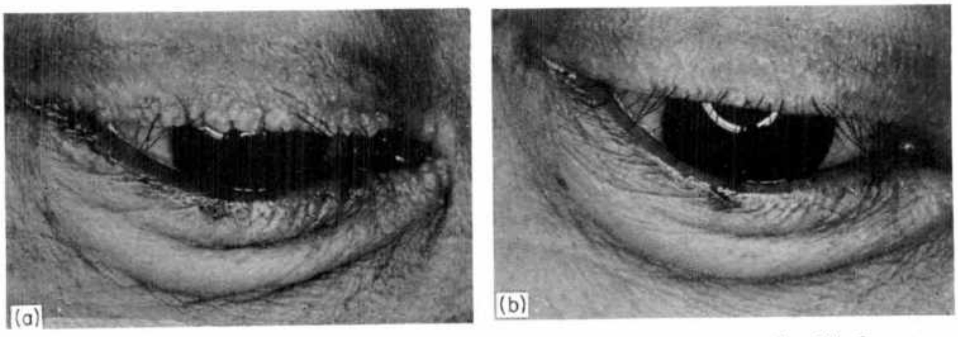

FIGURE 2. (a) Skin lesions on the left eyelid margin before treatment. (b) Appearance of eyelid after 3 years treatment with dimethyl sulphoxide.

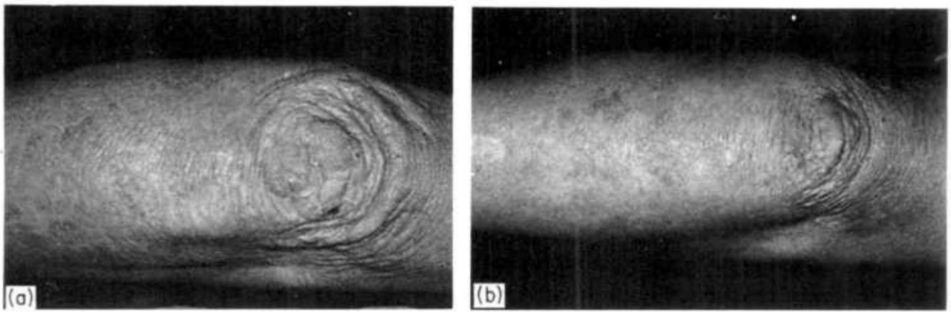

FIGURE 3. (a) Skin lesions on left elbow and forearm before treatment. (b) Appearance after 3 years treatment with dimethyl sulphoxide.

The patient's skin lesions improved markedly after 3 years of oral dimethyl sulphoxide therapy. The 'beaded' papules along the eyelid margins almost disappeared (Fig. 2). The hyperkeratotic papules and verrucoid plaques over the fingers, extensor aspects of the forearms, elbows, axillae, shins and perineum became either flattened or smaller (Fig. 3). The yellowish-white patches on the mucous membranes of the mouth and upper respiratory tract also improved. The patient's voice became clearer than it had been before therapy.

Repeat haematological, urinary and liver function tests, carried out every 3 months, showed no abnormality. A follow-up electrocardiogram was normal. The total mean oesophageal transit time was 36·8 s before dimethyl sulphoxide therapy, but fell to 5·78 s after 3-years of treatment (normal value: < 10 s).

The only side-effect was a garlic-like smell on the patient's breath.

DISCUSSION

Dimethyl sulphoxide is a dipolar aprotic substance which has exceptional solvent properties for both organic and inorganic chemicals. Its reported actions include membrane penetration, anti-inflammatory effects, local analgesia, weak bacteriostasis, diuresis, vasodilatation, and dissolution of collagen[4] and amyloid.[5,6] The toxicity of dimethyl sulphoxide appears to be low and the lens changes seen in animals after dimethyl sulphoxide have not occurred in humans.[4]

The beneficial effect of dimethyl sulphoxide on amyloidosis may be related, at least in part, to its ability to scavenge hydroxyl radicals.[7]

The hyaline material in lipoid proteinosis consists of excessive amounts of matrix glycoproteins, decreased quantities of collagen fibres and a general over-hydroxylation of all collagens. The changes of collagen metabolism revealed accumulation of type IV and V collagens around the vascular and appendageal areas, abnormally distributed type III collagen and a reduction in type I and III collagens. Thus, lipoid proteinosis involves a primary perturbation of collagen metabolism.[8]

Since dimethyl sulphoxide has been claimed to have the ability to dissolve collagen and scavenge hydroxyl radicals, we tried using this compound to treat our patient. His cutaneous symptoms, hoarseness of voice and abnormal oesophageal function all showed significant improvement without serious side-effects. This indicates that dimethyl sulphoxide can cause regression of hyaline deposits in lipoid proteinosis.

To the best of our knowledge, no other medication is known to improve the signs and symptoms of lipoid proteinosis satisfactorily. However, we do not know whether recurrence will be seen after discontinuation of oral dimethyl sulphoxide. We hope that our experience will encourage further therapeutic trials of dimethyl sulphoxide in cases of this disease.

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Intercellular IgA deposition in patients with clinical features of subcorneal pustular dermatosis

MADAM, In our previous report,[1] we described a patient with clinical features of subcorneal pustular dermatosis associated with intercellular deposition of IgA. Recently, Hashimoto *et al.*[2] reported the presence of circulating anti-intercellular IgA-class antibodies in sera from a patient with similar clinical features. We recently detected IgA-class antibodies against the intercellular material of the upper epidermis in sera from our previously described patient on a recurrence of the disease. We would like to report the new laboratory findings in this patient, together with the results of an immunoelectron microscopic study on the *in vivo* binding sites of the intercellular IgA antibodies.

A 58-year-old woman was hospitalized because of a vesiculo-bullous eruption on erythematous plaques on the axillae, trunk and limbs. A diagnosis of a variant of subcorneal pustular dermatosis had been made 8 years earlier and she had been treated successfully with etretinate 10 mg on alternate days for 8 years. The skin lesions, however, recurred, without obvious provoking factors, on the axillae and chest, and quickly spread over the trunk and limbs forming vesicles, pustules, and bullae on erythematous plaques. Laboratory tests showed the following abnormal values: white blood cell count, $17{\cdot}1 \times 10^9$/l (normal range, $3{\cdot}5$–$8{\cdot}0 \times 10^9$/l); lactate dehydrogenase, 441 U/l (normal, 75–320 U/l); glutamic-oxaloacetic transaminase, 92 U/l (normal, 8–30 U/l); glutamic-pyruvic transferase, 37 U/l (normal, 5–25 U/l); and serum IgA level, 870 mg/dl (normal 37–428 mg/dl) with a monoclonal peak on immunoelectrophoresis. On her previous admission serum IgA was 470 mg/dl without a monoclonal peak.

Biopsy specimens showed subcorneal pustules associated with infiltration of neutrophils and a small number of eosinophils in the epidermis and dermis. A few acantholytic cells were observed in the granular layer (Fig. 1a). Direct immunofluorescence using biopsy specimens of perilesional skin confirmed our previous observation[1] of intercellular deposits of IgA in the upper 3–4 layers of the epidermis without any

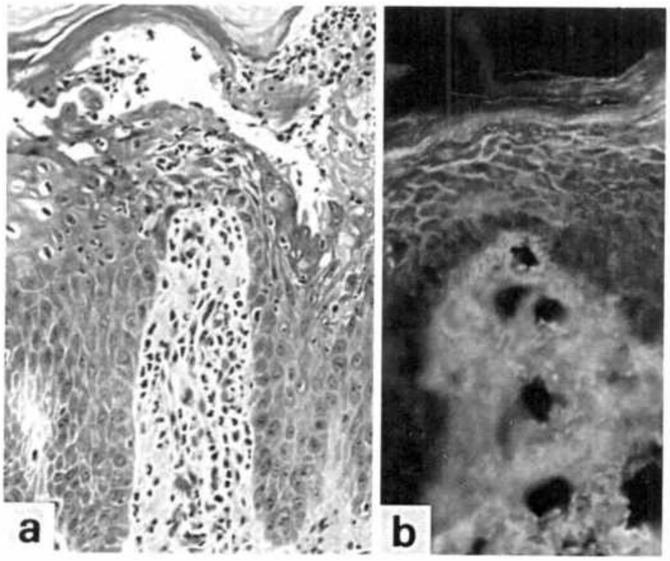

FIGURE 1. (a) Subcorneal blister formation with infiltration of neutrophils and a small number of eosinophils. Note the presence of a few acantholytic cells (H & E, original × 150). (b) Intercellular IgA deposition demonstrated by indirect immunofluorescence using a partially purified immunoglobulin fraction.

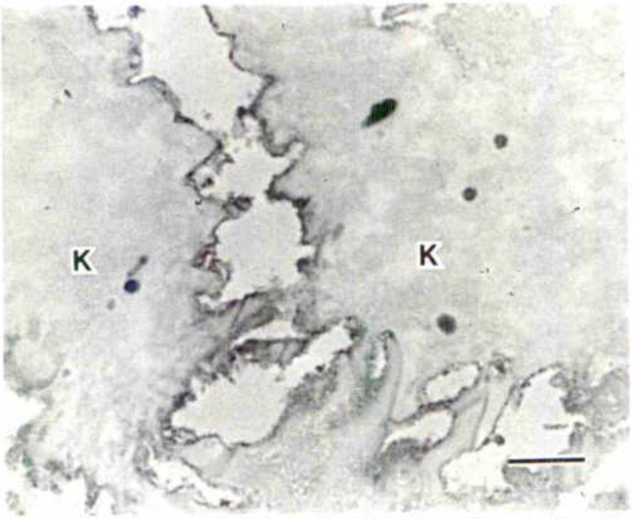

FIGURE 2. *In vivo* bound IgA detected on the surface of two keratinocytes (K) (unstained section; bar = 0·1 μm).

evidence of deposition of IgG, IgM or C_3. Indirect immunofluorescence, unlike our previous findings,[1] demonstrated the presence of circulating IgA-class antibodies against normal human epidermis in undiluted sera. No specific fluorescence was found with five normal sera and five sera taken from patients with psoriasis vulgaris. Using an immunoglobulin fraction that had been partially purified and concentrated by an ammonium sulphate precipitation method, we succeeded in demonstrating clear IgA deposition in the upper layer of the epidermis with less background fluorescence. The fluorescence pattern was very similar to that obtained with direct immunofluorescence (Fig. 1b). Neither IgG- nor IgM-class antibodies bound to the intercellular spaces of the epidermis.

Biopsy specimens of perilesional skin, fixed in 2% paraformaldehyde were reacted with peroxidase-labelled goat anti-human IgA antibody, and processed for immunoelectron microscopy.[3] Ultrathin sections were observed in an electron microscope with or without counterstaining. Ultrastructurally, the reaction products were specifically detected on the surface of the keratinocytes, encircling the cell membrane (Fig. 2). Both desmosomal and non-desmosomal areas were stained in a similar manner.

The patient was treated with dapsone, 75 mg daily, with a favorable clinical response. The elevated serum IgA level decreased to 517 mg/dl and the other abnormal test results normalized within a month.

Subcorneal pustular dermatosis is known to be associated with IgA gammopathy or pyoderma gangrenosum or both.[4,5] Interestingly, some patients have shown IgA deposition in the epidermis, especially in the upper portion. In our previous report,[1] we described our case as a variant of subcorneal pustular dermatosis showing a vesiculo-papular eruption with subcorneal deposition of IgA. Recently, Huff *et al.*[6] have reported a patient with similar clinical and laboratory features, designating it intraepidermal neutrophilic IgA dermatosis. Our case and the cases later reported by Hashimoto *et al.*[2] and by Huff *et al.*[6] may share similar immunological abnormalities closely linked to intercellular IgA deposition.

The present study, which gave similar results to those of Hashimoto *et al.*,[2] clearly demonstrated the presence of circulating IgA class antibodies directed against intercellular material in the epidermis. Use of a sensitive method or the concentration and purification of serum samples, as described in the present study, may be required to detect antibodies at titres lower than the level detectable by conventional indirect immunofluorescence. Furthermore, on immunoelectron microscopy, the IgA-class antibodies were found to bind to the surface of differentiated keratinocytes. We believe that detailed study of the pathogenetic role of intercellular IgA deposition may be of value in categorizing patients with the clinical features of subcorneal pustular dermatosis.

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Decreased stratum corneum ceramides in atopic individuals—a pathobiochemical factor in xerosis?

MADAM, The presence of generalized dry skin is a characteristic feature of atopic dermatitis which fluctuates with disease activity. Besides xerosis, ichthyosis has been reported from 2–6% of patients with atopic dermatitis. Intolerance of lipid solvents is another common feature of atopic skin.[1] These symptoms correlate with the disturbed water permeability barrier and diminished hydration of atopic skin.[2–4]

At an ultrastructural level the water permeability barrier is represented by the intercellular multilamellar lipid sheets of the stratum corneum.[5,6] Biochemical characterization of the lipid content of the lamellar bodies (membrane-coating granules) confirmed the electron-microscopic observation[7] that the intercellular barrier lipids of the stratum corneum are derived from exocytosis of lamellar bodies.[8,9] Under physiological conditions the lamellar bodies are filled with stacks of discoid lipid lamellae. After exocytosis into the intercellular space of the stratum corneum, these discs are rearranged parallel to the apical cell membranes of the granular cells.[7] The major lipid constituents of the intercorneocyte lipid domains are ceramides, a unique heterogeneous group of sphingolipids. A functionally most important acylceramide consists of a sphingosine base with an amide-lined long-chain ω-hydroxy acid, and an ester-linked nonhydroxy acid, 41% of which has been shown to be linoleic acid.[10] This linoleate-rich acylsphingolipid appears to serve as a molecular rivet in locking together the multilamellar lipid sheets in the stratum corneum.[6] In essential fatty acid deficiency, linoleate is replaced by oleate in these O-acylsphingolipids leading to formation of defective lamellar granules which lack the lamellate internal structure. This ultrastructural defect is associated with disintegration of the intercorneocyte lipid domains leading to increased transepidermal water loss.[11,12] Recently, it has been reported that lamellar bodies of 'dry' non-eczematous skin of patients with atopic dermatitis exhibit a greater volume, indicating a disturbance of lamellar body maturation. The ultrastructural abnormality seems to be related to the defect in barrier function in atopic skin.[13] Furthermore, similar changes in lamellar bodies lacking the internal discoidal lipid lamellae have been observed in xerosis senilis.[14] From solvent challenge experiments on human stratum corneum it has been recognized that the water-holding properties of the stratum corneum predominantly depend on the presence of ceramides.[15]

Therefore, we have analysed stratum corneum lipids of untreated non-inflamed plantar skin of 10 patients with atopic dermatitis and 10 healthy age-matched controls by sequential high-performance thin-layer chromatography.[16] A significant decrease in the ceramide fraction, expressed as a percentage of total lipid, was found in the patients with atopic dermatitis (mean ± SEM, 29·0 ± 3·6%) compared with the controls (35·0 ± 3·2%) ($P<0{\cdot}001$; paired t-test).

These findings point to impaired ceramide biosynthesis in patients with atopic dermatitis, resulting in xerosis with increased transepidermal water loss and diminished water-holding capacity of the stratum corneum. The deficient ceramide biosynthesis might be stimulated by administration of excess amounts of linoleate for synthesis of O-linoleylsphingolipids, a possible explanation for the beneficial effect of oral and topical treatment with essential fatty acids.[17-21]

It is possible that topical treatment with linoleate-rich sphingolipids might improve the skin condition in atopic individuals.

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Failure to demonstrate the lupus anticoagulant in systemic sclerosis

MADAM, Systemic sclerosis is a multisystem disorder, usually presenting with Raynaud's phenomenon, in which vascular changes and later connective tissue fibrosis occur in the skin and internal organs. In view of the fact that thrombosis can be a feature, the blood of 24 unselected patients with systemic sclerosis fulfilling the criteria of the American Rheumatism Association[1] was examined for the presence of the lupus anticoagulant using the activated partial thromboplastin time (APTT) and kaolin clotting time (KCT).[2]

The lupus anticoagulant could not be demonstrated in any of the patients. None of the patients had a positive VDRL test.

Our failure to demonstrate the lupus anticoagulant by the APTT and KCT could be due to the insensitivity of the techniques. The Russell viper venom time (RVVT) has been claimed to be a more sensitive index of the lupus anticoagulant,[3] but we consider that the Manchester standardized APTT[2] is a very sensitive test and the APTT and RVVT do not always agree.

The lupus anticoagulant is only one of several antiphospholipid antibodies, and anticardiolipin antibodies (ACA) at low concentrations have been found in 11 of 35 patients with systemic sclerosis,[4] although there was no correlation with any clinical manifestations such as ischaemic ulceration, peripheral venous disease, interstitial lung disease or pulmonary hypertension. Tests on our patients with systemic sclerosis have shown anticardiolipin antibodies in 25%, particularly in those with extensive visceral disease.[5] As these patients frequently had circulating immune complexes and raised levels of von Willebrand factor antigen, it is possible that antiphospholipid antibodies such as ACA may play some part in the pathogenesis of the vascular abnormalities of systemic sclerosis.

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Loss of antibiotic activity caused by photodegradation: *in vivo* studies

MADAM, It is well-known that many drugs significantly absorb wavelengths of radiation within the solar spectrum. Some dissipate this absorbed energy by a variety of mechanisms damaging to the skin and so produce clinical photosensitization. The majority of these reactions are toxic in mechanism (phototoxic), while others involve a sensitized immune process (photoallergic). Following absorption of radiant energy, photoproducts, which are locally[1] or systemically toxic[2] may be produced.

The pharmaceutical industry has for some time been aware that drugs may undergo photodegradation when stored in clear glass bottles. Consequently, it is generally recommended that amber glass containers be used for drug dispensing.[3]

To our knowledge, the effect of irradiation on the therapeutic effectiveness of a drug has not been studied. In particular, no data exist concerning the wavelength and radiation dose dependency of such an effect nor the relationship to environmental radiation exposure in a patient taking the drug. These considerations give rise to the following question. Is a patient who takes a drug which strongly absorbs radiation, and who is exposed environmentally to that radiation in the form of sunbathing, sunbeds, phototherapy, or photochemotherapy, at risk of reducing the therapeutic effectiveness of that drug?

Although there are no clinical reports of cutaneous photosensitivity due to ciprofloxacin, like other members of the fluorinated quinolone group,[4,5] it is known not only to absorb strongly in the UVA spectrum, but also appears to have *in vitro* phototoxic properties.[6] We have now used a standard bacteriological bioassay to establish wavelength and radiation dose dependency data in a study designed to determine the possibility and extent of ciprofloxacin inactivation by exposure to sunlight whether natural or artificial.

Samples of ciprofloxacin in plasma (0·25 ml; 8 mg/l) were exposed to one of three radiation sources at a radiation dose within the range of environmental exposure: UVB (Phillips Colour 12; 290–350 nm; 1500 mJ/cm^2), UVA (Sylvania PUVA lamp; 320–430 nm; 1 J/cm^2 and 10 J/cm^2) and visible (Xenon arc solar simulator Schott GG425 filter; 977 J/cm^2). The concentration of antibiotic in irradiated and non-irradiated control plasma samples was measured using a standard agar well diffusion technique with *E. coli* NCTC 10418 as the indicator organism. All experiments were repeated on five occasions.

The results (Table 1) showed that, even when used at high dosage, visible radiation sources failed to reduce the antibiotic effect of the ciprofloxacin containing plasma sample. However, UVA at 10 J/cm^2 did significantly reduce the antibiotic effect and, in addition, a less marked effect was noted with 1 J/cm^2. With

TABLE 1. Concentration of ciprofloxacin in plasma (mg/l)

	Irradiated samples (J or mJ/cm^2)				
No irradiation	1500 mJ UVB	0·5 J UVA	1·0 J UVA	10 J UVA	Visible
8·4	5·2	7·4	6·0	1·7	7·7
7·6	5·5	7·9	6·8	1·6	7·6
8·0	6·2	7·8	7·5	2·0	8·0
7·7	5·4	7·3	8·0	2·1	7·0
7·4	4·7	7·6	6·3	2·1	7·9
Mean 7·82	5·40	7·60	6·92	1·90	7·64
SD 0·39	0·54	0·25	0·83	0·23	0·39
t	9·929*	0·943	2·038	25·152	0·779

* Test statistic exceeds the critical value of t corresponding to a nominal significance level of 0·001, or an overall level of 0·005 using a Bonferroni adjustment to allow for the fact that 5 hypotheses are being tested.

the UVB source, at the exposure dose used, an effect was obtained which was slightly greater than that for 1 J/cm^2 of UVA. However, this UVB dose would produce a severe sunburn reaction in the majority of normal subjects and the emission from the source contains significant amounts of UVA.

It is evident that UVA wavelengths, in significant amounts, penetrate the dermis where 20% of the cardiac output is directed. Because sunbeds and sunbathing may expose patients taking ciprofloxacin to more than 10 J of UVA in a day (UVA at noon in midsummer in Dundee is approximately 5 mW/cm^2, i.e. 18 J/cm^2/h), it is possible that environmental exposure to these wavelengths may result in a reduced antibiotic effect of this drug. The preliminary results reported here are being followed up by precise monochromatic wavelength dependency studies, as well as pharmacokinetic work to determine their significance *in vivo*.

The photochemical change in ciprofloxacin may be monitored very simply by following the UV-induced change in its absorption spectrum or, with more sophistication, using high performance liquid chromatography (HPLC). Our own studies with HPLC have revealed a number of photoproducts. However, until these compounds have been identified and their own antibiotic potential determined, the bioassay remains the main indicator of UV-induced loss of therapeutic activity. The role of photoproducts, as potential local and systemic toxic agents, also requires further study. It may well be that simple avoidance of irradiation or use of appropriate photoprotection will minimise both the toxicity and the possible loss of therapeutic activity.

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Etretinate-responsive pachyonychia congenita

MADAM, We were interested to read the recent correspondence regarding the treatment of pachyonychia congenita.[1,2] Unlike Drs Soyuer and Candan[1] we have seen definite improvement in a patient with type I pachyonychia congenita following treatment with etretinate.

A 46-year-old man was first seen at St John's Hospital in 1964 at the age of 22. His finger nails and toe nails had been long and thickened since infancy, the latter becoming wedge-shaped (Fig. 1). When 4 years old he was troubled by recurrent blistering and crust formation on the soles of his feet. This was followed by the gradual development of hyperkeratosis of the soles (Fig. 2), accompanied by profuse sweating and a pungent odour. His tongue later became white and fissured. Painful fissuring of the soles made walking very difficult without the aid of a walking stick. There was no family history of pachyonychia congenita.

A number of topical keratolytics were tried without success. In 1980, etretinate therapy was commenced at 25 mg/day (body weight 68 kg) and this was increased to 50 mg daily after 2 weeks. After 3 months of

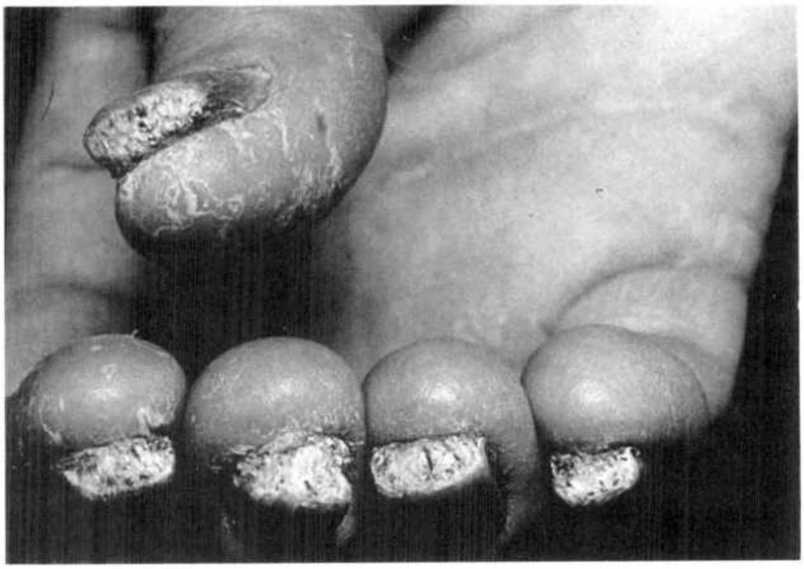

FIGURE 1. Wedge-shaped nails in pachyonychia congenita.

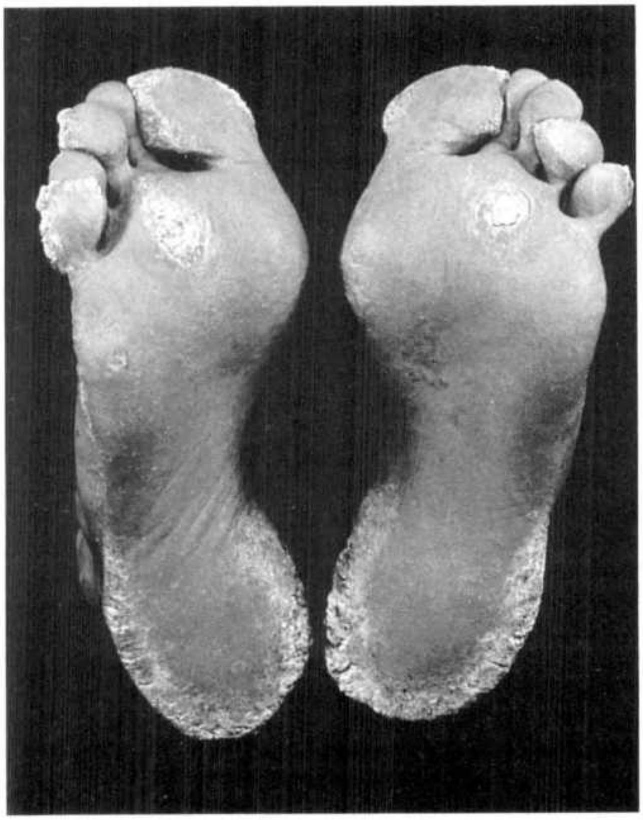

FIGURE 2. Hyperkeratosis of the soles in pachyonychia congenita.

therapy there was a reduction in the amount of hyperkeratosis on the soles, and he was able to walk comfortably without the use of his walking stick. After a further 2 months of treatment, he walked 5 miles for the first time in his life. Temporary discontinuation of the etretinate during the past 8 years has been followed by exacerbation of his symptoms. X-rays of the vertebral column have revealed minor degenerative changes only and he remains improved on etretinate.

Thus, our patient experienced marked symptomatic improvement with decreased hyperkeratosis following 3–5 months of etretinate therapy. It may be that the patient described by Drs Soyuer and Candan would have responded to more prolonged treatment with etretinate, had the drug been tolerated for more than 5 weeks.

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The suitability of sunglasses worn by PUVA patients

MADAM, In a recent paper (*British Journal of Dermatology* 1988; **118:** 247), Dr Moseley and colleagues advised against the practice of checking the optical suitability of sunglasses worn by PUVA patients by using UVA radiation from the opened door of the PUVA cubicle and the departmental UVA meter. I examined this practice critically some time ago[1] and concluded that this simple technique was unlikely to lead to a misleading impression concerning the ultraviolet opacity of the patients' suglasses in natural sunlight. Given that a spectrophotometer is not readily to hand in most PUVA departments, I commend this quick and simple checking procedure to staff as a worthwhile alternative to spectral transmission measurements.

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REFERENCES

1 Diffey BL, Miller JA. A comment on the routine testing of sunglasses in photochemotherapy. *Br J Dermatol* 1980; **102:** 665–8.

Reply

MADAM, Dr Diffey raises an interesting point in his letter, which we are pleased to comment on. The reasons for our advising against dermatologists placing undue reliance on the procedure described by Dr Diffey are as follows: Firstly, the 'transmission' so measured will vary depending on the spectral emission of the UVA lamps and the spectral sensitivity of the UVA meter. Secondly, PUVA lamps usually have a peak emission at a wavelength of around 360 nm. However, we have suggested that shorter wavelengths are likely to be more important for PUVA-induced cataract from daylight exposure. If this is the case, then UVA lamps and a UVA meter would be measuring 'transmission' in the wrong part of the spectrum. Thirdly, we have examined eyewear where transmission peaks occurred within the UV region. For example, one pair had a transmission of 5% of 400 nm and 15% at 340 nm. A UVA lamp-UVA meter combination would not give a reliable indication of the suitability of eyewear in such cases. The suitability of sunglasses worn by PUVA patients is an important issue, and should perhaps be considered by the British Association of Dermatologists.

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Polymorphic light eruption sine eruptione and brachioradial pruritus

MADAM, In describing polymorphic light eruption sine eruptione (PLESE) (*British Journal of Dermatology* 1988; **118:** 73) Drs Dover and Hawk appear to have overlooked a condition recently reviewed in your journal.[1] Brachioradial pruritus (BRP) is still to be clearly defined, but many patients with BRP have a pronounced aggravation of pruritus following sun exposure, without developing a skin eruption. It is most commonly recognized in 'the tropics'. (Sydney presumably meets this climatic criterion judging by the incidence of BRP amongst my patients).

The symptoms of BRP usually begin in adult life and can be an itch, burning prickle or a feeling of 'pins and needles'. Sites of the body other than the forearms can be involved. Patients with BRP often learn not to complain to unaware doctors, a course also followed by patients with aquagenic pruritus.

In my experience the phenomenon of summer 'hardening' does not occur in BRP. I would be interested in the views of Drs Dover and Hawk on a possible overlap between PLESE and BRP. Could some cases of PLESE be called brachioradial pruritus in climates where there is prolonged and intense sun exposure?

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1 Walcyk PJ, Elpern DJ. Brachioradial pruritus: a tropical dermopathy. *Br J Dermatol* 1986; **115:** 177–80.

Reply

MADAM, Dr Commens has asked us to compare the features of brachioradial pruritus (BRP) with those of our polymorphic light eruption sine eruptione (PLESE). The comparison is interesting, but I do not think that BRP and PLESE are related. PLESE affects patients with a similar age range and sex ratio to those with polymorphic light eruption (PLE), occurs on widespread areas of the body in a similar fashion to PLE and is not associated with xerosis or excoriations. BRP occurs in an older age group and equally in both sexes, is only on the flexor surfaces of the elbows, is usually accompanied by xerosis and there are often excoriations. The condition seems likely to be a manifestation of chronic solar damage to a constantly exposed site, whereas PLESE seems to be a forme fruste of PLE.

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British Journal of Dermatology (1988) **119,** 555–556.

Book Reviews

Common Problems in Dermatology. Edited by K.E.Greer. Year Book Publications. Pp. 455. ISBN 0-8151-3561-0. Price £41.50.

Current Therapy in Dermatology, 2. Edited by T.T.Provost and E.R.Farmer. Toronto: B.C. Decker Inc. Pp. 293. ISBN 1-55664-023-4. Price £38.00.

To be asked to review these two volumes together makes good sense, as they have very similar aims and layouts.

The text by Greer is problem orientated and contains 64 scenarios where Dr Greer has given appropriate experts a management problem relating to common problems, such as scabies and acne, and to rarer problems, such as panniculitis and Kaposi's sarcoma.

Current Therapy in Dermatology is a similar layout in which 97 authors have been asked to write on the management of diseases ranging from sunburn to leprosy. There is an overlap of five authors in the two books, but none have been asked to write on the same problem twice.

I personally found the problem orientated layout of Greer rather more 'user friendly', and found a number of the scenarios, such as that on atopic dermatitis dealt with by Hanifin, very similar to problems encountered in my own practice. In the Provost and Farmer text, a particularly good article is that on acquired epidermolysis bullosa management by Caughman.

My only problem about both these texts is that I wonder whether or not the individual who requires to look at such a text, for example on the management of say scabies or warts, is also going to require the text for the management of AIDS or advanced cutaneous lymphoma. While both are valuable, it might have been better to have had two texts divided up, one into basic therapy aimed more at the primary care practitioner, and one into more specialised therapy aimed at the dermatologist.

Both texts are good value for money, and the individual choice for purchase will depend on personal preference. I would recommend a quick browse through both before a purchase decision is made.

Rona M.MacKie

Atlas of Dermatopathology. R.P.Rapini and R.E.Jordon (1988) Chicago: Year Book Publishers. Pp. 414. Price £65.00.

The aim of this new book is to provide quickly the important histological criteria and a comprehensive differential diagnosis for a given disease or histopathological feature. Clinical details and pathogenesis of the diseases are specifically not included.

The first chapter provides a comprehensive list of pathological changes that one might encounter in a skin biopsy, e.g. presence of multinucleate giant cells, and enumerates, with cross references, all the situations where they might arise. Subsequent chapters describe the histological features of individual skin diseases in note form with a cross referenced list of differential diagnoses. Interspersed between the notes are 461 black and white labelled photographs of average to good quality. In addition, there are 102 colour plates of good quality overall, but a few suffer in impact because they are either at too low or too high a magnification. However, many special stains are demonstrated in colour, including immunofluorescence.

Certainly the aims of the authors are more than achieved. The book provides a convenient quick reference check for the busy dermatopathologist, but I suspect it will be used most by trainees in both pathology and dermatology as well as dermatopathology. As such, if left by the microscope, it would be a well-used departmental addition, but as a supplement, not a substitute for the larger histopathology texts.

D.H.McGibbon

Books Received

Advances in Dermatology, Vol. 3. Edited by J.P.Callen, M.V.Dahl, L.E.Golitz, L.A.Schachner and S.J.Stegman (1988) Chicago: Year Book Medical Publishers. Pp. 318. Price £37.00.

Common Skin Disorders. A Physician's Illustrated Manual with Patient Instruction Sheets. E.Epstein. 3rd edition (1988) Oradell, New Jersey: Medical Economics Books. Pp. 200 + 110. Price $35.95.

Leprosy for Medical Practitioners and Paramedical Workers R.H.Thangaraj and S.J.Yawalkar. 3rd edition (1988). Pp. 108. Distributed free of charge by Ciba-Geigy Ltd, CH-4002, Basle, Switzerland.

British Journal of Dermatology (1988) **119**, 557.

News and Notices

Society for Cutaneous Ultrastructure Research Annual Meeting

The 16th Annual Meeting of the Society for Cutaneous Ultrastructure Research (SCUR) will be held in Köln, West Germany, on 9–10 June 1989. For further information contact: Professor Dr Gustav Mahrle, Department of Dermatology, University of Köln, Joseph-Stelzmann-Str. 9, D-5000 Köln 41, Germany. Tel: 01049-221-478-5086.

Mersey Regional Council for Postgraduate Medical Education, University of Liverpool

5th 2-Day Course in Paediatric Dermatology

The 5th 2-day Course in Paediatric Dermatology will be held at the University of Liverpool, U.K. on 11–12 May 1989. The course is for consultants, senior registrars and registrars recently appointed in dermatology, and is limited to 12 participants. Full details available from: Dr J.L.Verbov, c/o Postgraduate Dean's Office, Faculty of Medicine, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K. Tel: 051-709-3114.

The 4th International Symposium on the Treatment of Psoriasis and Psoriasis-arthritis

The 4th International Symposium on the Treatment of Psoriasis and Psoriasis-arthritis will be held in Jerusalem from 5–12 March, 1989. For further information contact Health Vacation Center, P.O.B. 99, Ramat Hasharon 47100, Israel.

British Journal of Dermatology (1988) **119**, 558.

Erratum

In the summary of Poster No. 18 in the BAD Annual Meeting Supplement: The contribution of direct immunofluorescence (DIF) to the diagnosis of lupus erythematosus, R.E.A.Williams, R.O'Keefe, R.M.MacKie and W.Thomson, (1988; 119, Supplement 33, page 52), the last paragraph should have read as follows: DIF was most useful in the diagnosis of SLE; when a diagnosis of CDLE or a request to exclude LE was made, DIF should not be performed until the results of light microscopy and serology are known.

British Journal of Dermatology (1988) **119**, 559–565.

Effect of BANS location on the prognosis of clinical stage I melanoma: new data and meta-analysis

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SUMMARY

Intermediate thickness (0·76–1·69 mm) clinical stage I melanomas at BANS locations (upper Back, posterior Arm, posterior Neck, posterior Scalp) were initially reported to have worse prognosis than similar thickness lesions at other subsites. Subsequent investigation did not support this observation. We re-examined the hypothesis in 156 consecutive patients with clinical stage I melanomas in this thickness range, who were seen at the Massachusetts General Hospital from 1977 to 1985. Patients with BANS lesions suffered recurrences at twice the rate of those with non-BANS lesions (28% compared with 13% at 5 years after excision of the primary melanoma), but this difference was not statistically significant.

A meta-analysis of the effect of BANS subsite on death risk was performed using data from our investigation and five comparable published studies. The original data set which led to the BANS hypothesis was excluded from the meta-analysis in order to avoid bias. BANS location was associated with a 60% increased risk of death ($P = 0{\cdot}002$). The difference remained statistically significant after elimination of any two of the six studies from the analysis. This confirms the influence of anatomical subsite on the prognosis of intermediate thickness melanoma.

In 1982, Day *et al.*[1] reported a relatively higher risk of death from melanoma in patients with primary tumours located on the upper Back, posterior Arm, posterior Neck, and posterior Scalp (BANS). That study group consisted of 203 patients with clinical stage I melanoma and primary tumours 0·76–1·69 mm thick, who were evaluated between 1 September 1972 and 30 May 1977

Part of this work was presented at the Society for Investigational Dermatology Regional Meeting, New York, NY, October 1986.

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at either New York University Medical Center or the Massachusetts General Hospital. Of the 67 patients with primary tumours located in one of the BANS locations, 11 (16%) died during the study. In comparison, only one (0·77) died among the 136 non-BANS patients. This difference was statistically significant ($P<0 \cdot 0001$).

Since this publication, several authors[2–6] have denied the prognostic importance of BANS location. In the present investigation we have re-examined the relation of BANS subsite to prognosis in a more recent group of melanoma patients followed at one of the original institutions, and we present a meta-analysis of the published literature on this question.

METHODS

The study population consisted of the 156 consecutive patients seen with 0·76 to 1·69 mm thick clinical stage I primary cutaneous malignant melanoma at the Massachusetts General Hospital pigmented lesion clinic from 1 June 1977 (the termination date of the Day *et al.*[1] patient group) to 31 December 1985. The clinic primarily serves the greater Boston area. A small percentage of patients come from the surrounding New England states.

The histology of the primary melanoma was evaluated by the Massachusetts General Hospital dermatopathology department staff. Each patient was entered into the study at the time of initial surgical treatment for the primary melanoma. This report includes follow-up through 1985.

Product limit estimates of recurrence risk were calculated by the method of Kaplan and Meier,[7] and their standard errors by Greenwood's formula.[8] The log-rank test and corresponding approximate sample size determinations employed the methods of Peto and Peto,[9] Freedman,[10] and Hsieh.[11] Cox proportional hazards regression was performed in the conventional manner.[12]

Meta-analysis refers to the review and combined re-analysis of multiple studies of the same research question. For the purpose of meta-analysis, publications which compared outcome of melanomas in BANS and non-BANS regions among patients in the 0·76 to 1·69 mm thickness group were reviewed. Reports which examined anatomical subsite of melanoma without specifically segregating the BANS sites were excluded, as were those which did not consider primaries less than 1·7 mm separately from thicker lesions. A list of appropriate studies was derived from the authors' knowledge of the field and a computerized search of the National Library of Medicine database from 1982 through to October 1987. This search included all articles on melanoma prognosis which mentioned location, region, site, or derivatives of these words in the abstract or title, as well as all articles on melanoma which mentioned the term 'BANS' in the abstract or title. Calculations for the meta-analysis were performed using the Mantel-Haenszel test statistic[13] and precision-based effect estimates for the relative risk of mortality.[14] Homogeneity of the above estimates was evaluated with the conventional chi-square test.[15] The relative risk of mortality is defined as the risk of death during the period of observation among patients with melanoma in the BANS areas divided by that risk among non-BANS patients.

RESULTS

Eighteen (12%) of the 156 patients suffered recurrences of the melanoma. Eleven of these recurrences were fatal. Three patients were alive with visceral metastases (one involving each of brain, spinal cord, and bone) and four were clinically disease free after resection of nodal (three cases) or in transit (one case) metastases. Two (1%) died of causes unrelated to the melanoma, and were clinically free of melanoma at the time of death. Among the 136 surviving recurrence-free patients, five (4%) were lost to follow-up prior to 1985. The mean duration of follow-up for the remaining 131 patients was 4·3 years (range 0·3 to 8·9 years).

The primary melanoma was located in the BANS region in 44 patients (28%). The BANS group had a significantly lower proportion of level IV lesions: 23%, compared with 44% in the non-BANS group ($P = 0{\cdot}02$). There were no other significant differences between the BANS and non-BANS groups with regard to any of the patient or tumour characteristics examined (see Table 1).

Of the 18 recurrences, eight (including six of the 11 fatal recurrences) were among the BANS patients. The difference in recurrence rate between the BANS and non-BANS groups was not statistically significant ($P = 0{\cdot}09$; log-rank test). The BANS group did have proportionately more recurrences, however: their 5-year recurrence rate (± SEM) was 28 ± 9%, twice that of the non-BANS group (13 ± 4%). Cox univariate proportional hazards regression confirmed the hazard ratio of 2·2, with a 95% confidence interval of 0·9 to 5·6. Neither this point estimate nor its variance were appreciably affected by controlling for age or level of invasion. There was no evidence of unequal censoring distributions for BANS ($P = 0{\cdot}6$; log-rank test).

TABLE 1. Patient characteristics according to the site of the primary melanoma

	BANS ($n = 44$)	Non-BANS ($n = 112$)	P
Level of invasion			0·02
II	3 (7%)	12 (11%)	
III	30 (68%)	48 (43%)	
IV	10 (23%)	49 (44%)	
V	0	0	
unknown	1 (2%)	3 (3%)	
Histological type			0·2 (nodular vs. superficial spreading)
superficial spreading	31 (70%)	84 (75%)	
nodular	7 (16%)	8 (7%)	
lentigo maligna	0	3 (3%)	
acral lentigenous	0	1 (1%)	
minimal deviation	0	1 (1%)	
unclassified	6 (14%)	14 (12%)	
unknown	0	1 (1%)	
Elective regional lymphadenectomy			0·8
performed	7 (16%) (all negative)	22 (20%) (one positive, 21 negative)	
not performed	37 (84%)	90 (80%)	
Sex			0·07
male	23 (52%)	39 (35%)	
female	21 (48%)	73 (65%)	
Age at diagnosis, years (mean ± SD)	43 ± 16	45 ± 14	0·4
Thickness, mm (mean ± SD)	1·16 ± 0·28	1·13 ± 0·26	0·5

Possible prognostic significance of other variables was assessed by Cox regression. No relationship was found between Clark's level and recurrence ($P=0{\cdot}5$, see Table 2), whether or not BANS was included in the regression model. Similarly, the following variables were unrelated to outcome: thickness ($P=0{\cdot}5$), sex ($P=0{\cdot}6$), node dissection performed ($P=0{\cdot}7$), age ($P=0{\cdot}11$), and histological type ($P=0{\cdot}9$).

The broad confidence interval of the hazard ratio for BANS led us to estimate the number of patients needed to confidently detect a difference between 15% and 30% recurrence rates. We assumed that 30% of melanomas are in BANS sites and we set type 1 error at 5%. It follows (see methods) that approximately 348 patients need to be followed (i.e. approximately 68 recurrences observed) to have 80% power to detect the specified difference.

META-ANALYSIS

None of the published studies of the effect of BANS on prognosis in this thickness range reached the size required to reliably detect the differences in which we are interested. Hence, we performed a meta-analysis of this issue. Meta-analysis involves review and statistical combination of results from multiple studies of a specific hypothesis. Although not yet common in the dermatological literature, the meta-analytic approach is becoming increasingly important in other areas of medical research, particularly for improving effect estimation and statistical power.[16,17]

Table 3 lists the studies of this issue which met our criteria (see methods). All derive from hospital based melanoma registries, hence may be affected by referral patterns. The listed outcome variable in each case was the proportion of patients who died while under observation, because the published data did not allow adequate comparison of survival times or rates among the listed studies. The loss of efficiency resulting from this choice of outcome variable is likely to be small.[19]

The report of Day *et al.*[1] was included in Table 3, but excluded from all further statistical analysis, for the following reason. The BANS hypothesis was formulated by the authors of that study after observing the patterns in their data, and it had no a priori justification. Their estimates of effect were, therefore, biased towards extreme values; if their data were not dramatic, the BANS hypothesis might not have been formulated or published. In contrast, the subsequent studies specifically sought to test the previously proposed BANS hypothesis, so are free from this bias, and hence included in the analysis.

One additional study which evaluated the BANS hypothesis was excluded because the report did not specify the actual number of deaths in the BANS and non-BANS groups.[20] It stated that

TABLE 2. Recurrences in relation to Clark's anatomical level

Clark level	No. of patients disease-free	 with recurrences	5-year recurrence rate (± SEM)
II	13	2	17% (±11%)
III	71	7	14% (±5%)
IV	50	9	25% (±8%)

TABLE 3. Reports of survival in relation to BANS location in patients with clinical stage I melanoma

Reference	Thickness (mm)	Proportion dead				Relative risk	Deathrate		Notes
		BANS	(*n*)	non-BANS	(*n*)		BANS	non-BANS	
Blois *et al.*[2]	0·76–1·69	17%	(107)	11%	(195)	1·6	na	na	a
Cascinelli *et al.*[5]	0·76–1·69	28%	(29)	22%	(123)	1·3	31% (at 10 years)	33%	b
Day *et al.*[1]	0·76–1·69	16%	(67)	1%	(136)	22·3	na	na	
Kuehnl-Petzoldt *et al.*[18]	⩽1·5	23%	(83)	15%	(277)	1·5	na	na	c, d
Rogers *et al.*[6]	0·76–1·69	11%	(66)	6%	(145)	1·9	11% (at 5 years)	7%	
Woods *et al.*[4]	<1·69	3%	(74)	1%	(203)	1·8	3% (at 4 years)	na	
This report	0·76–1·69	14%	(44)	4%	(112)	3·1	24% (at 5 years)	7%	

na = not available

a Excluded acral lentigenous melanomas

b Included deaths from causes other than melanoma; 5-year death rates as follows:

thickness	BANS	non-BANS
<1·0 mm	11%	7%
1·1–2·0 mm	22%	17%

c This series consisted of 60 patients who died within 8 years of diagnosis and 300 patients who survived at least 8 years. Hence the 'proportion dead' does not directly measure actual death risk.

d The report did not specify the classification of deaths not due to melanoma.

the P-value of the survival comparison was 0·52, but it appears from their Figure 10 that there were only 58 patients analysed in this comparison, and only two or three of them had actually died.

The meta-analysis includes six studies which involve a total of 1458 patients and 173 deaths. BANS is associated with a significant excess of deaths in this pooled analysis (relative risk of mortality = 1·60, 95% confidence interval 1·19 to 2·15, P=0·002). The relative risk of 1·6 corresponds to a 60% excess risk of mortality. There was no evidence of heterogeneity among the six studies (P=0·9; chi-square test). Indeed, if any two of the studies were excluded from consideration, the excess risk associated with BANS location varied from 49% to 82% and remained statistically significant.

DISCUSSION

The question addressed by our analyses was whether BANS location is associated with poor prognosis in stage I melanoma patients with primary lesions 0·76 to 1·69 mm in thickness, as hypothesized by Day *et al.*[1] Examination of an independent series of 156 stage I melanoma patients demonstrated no significant difference between the BANS and non-BANS groups, yet our estimate of effect suggested that the rate of recurrence in the former group was double that of the latter. Hence the results are, by themselves, inadequate for assessing the question. Indeed, our calculations of statistical power suggested that the numbers required to answer the question were quite large.

Meta-analysis is indicated in precisely this circumstance. We excluded, for reasons discussed above, the original report by Day *et al.*[1] Although none of the studies reviewed found a statistically significant difference in prognosis between BANS and non-BANS patients, all noted a higher proportion of deaths in the BANS group. Four of the studies reported death rates; in three the death rates were higher in the BANS group. Cascinelli *et al.*[5] observed a 10-year death rate of 31% in BANS patients and 33% in non-BANS patients. However, the death rate in the BANS group was lower during years 3–9 after diagnosis, when the bulk of melanoma-induced mortality occurs in lesions 0·76 to 1·5 mm thick.[21] Since Cascinelli *et al.*[5] included all deaths, not just deaths from melanoma, one must consider the possibility that there was a greater death rate due to intercurrent illness in the non-BANS group, along with a greater death rate due to melanoma in the BANS group.

Meta-analysis is subject to possible 'publication bias'. This bias would occur here if studies which found a statistically significant difference between BANS and non-BANS sites were more likely to be published than those which found no difference. However, since none of the studies which followed Day *et al.*[1] found a statistically significant difference between the BANS and non-BANS groups, and each of the studies which addressed the BANS hypothesis was reported as refuting it, 'publication' bias towards extreme results is quite unlikely here.

Meta-analysis revealed a 60% elevation of death risk in the BANS patients, which was statistically significant. This result verifies the hypothesis that anatomical subsite, and BANS location in particular, effects prognosis of 0·76 to 1·69 mm thick clinical stage I melanoma.

The prognostic importance of location may differ among thickness groups, and there are categorizations of anatomical subsites other than the BANS/non-BANS dichotomy. In most analyses of these alternative categories, melanomas from all thickness ranges are included. Balch *et al.*[3] presented evidence that the scalp and the feet are associated with poor prognosis, and the remainder of the extremities are associated with improved prognosis. Rogers *et al.*[22] suggested that high risk may be associated with lesions of the scalp, mandibular area, middle of trunk (anterior and posterior), upper medial thighs, hands, feet (except the arches), popliteal fossae and genitalia. Many other investigators have addressed the influence of anatomical site on prognosis. Although there are many differences among the conclusions of these studies, the extremities other than the hands and feet are often found to be associated with a better outcome than trunk lesions when all thickness groups are included.[23-26] Inconsistencies among the studies to date indicate the need for additional investigation.

The basis for differences in prognosis among anatomical subsites remains a matter of speculation. It may be related to vascular supply, lymphatic drainage, immune responsiveness, or other factors. An improved understanding of the role of anatomical subsite may lead to better management of primary lesions of melanoma. The present verification of the adverse prognosis associated with BANS location is a step in this direction.

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Gamma-interferon induced human keratinocyte HLA-DR synthesis: the role of dermal activated T lymphocytes

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SUMMARY

Experiments were performed to examine the hypothesis that the surface expression of HLA-DR by keratinocytes in certain disease states is conferred by the activity of gamma-interferon, derived from dermal activated T lymphocytes. *In vivo* studies revealed a spatial relationship between keratinocyte HLA-DR expression and activated T lymphocytes within the dermal inflammatory infiltrate. *In vitro* studies confirmed that gamma-interferon can induce keratinocyte synthesis of HLA-DR. These results suggest that, *in vivo*, gamma-interferon produced by activated T lymphocytes induces keratinocyte HLA-DR synthesis and expression.

Class I major histocompatibility complex (MHC) antigens are expressed on the cell surface of all adult nucleated cells. Class II MHC antigens, on the other hand, are expressed only on the surface of certain immunologically active cells such as B lymphocytes, activated T lymphocytes, macrophages and Langerhans cells. However, aberrant expression of HLA-DR has been observed on the surface of many different epithelial cell types in certain diseases such as thyrocytes in Graves' thyroiditis[1] and gut epithelium in graft-versus-host disease.[2]

Aberrant expression of HLA-DR by human keratinocytes has also been described in a wide range of dermatoses,[3,4] but most consistently in lichen planus,[5] cutaneous T cell lymphoma,[6] discoid lupus erythematosus,[4] allergic contact dermatitis[7–9] and cutaneous graft-versus-host disease,[10] each of which is characterised by a prominent dermal lymphocytic infiltrate. Lymphocytes produce a number of lymphokines including gamma-interferon (γ-IFN), which is synthesized and secreted by activated T lymphocytes.[11] It has many immunomodulatory functions including promotion of increased cell surface expression of class I MHC antigens,[12] activation of macrophages,[13] and induction of HLA-DR on certain cell types which do not usually express HLA-DR, including keratinocytes.[14,15] This has led to the hypothesis that

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γ-IFN released from dermal infiltrating cells may be responsible for the induction of HLA-DR expression by keratinocytes[16] in these diseases.

Since γ-IFN is a soluble mediator, diffusion into the epidermis would permit induction of keratinocyte synthesis of HLA-DR. This implies that keratinocyte HLA-DR expression should be maximal overlying zones of dermal infiltration by activated T lymphocytes. Activated T lymphocytes express a number of surface antigens not present on resting helper T cells including HLA-DR,[17] transferrin receptor[18] and interleukin 2 (IL − 2) receptor.[19] While HLA-DR is seen on many differing immunologically active cells and transferrin receptor is present on other proliferating cell types,[20,21] IL − 2 receptor is only present on activated T lymphocytes and sparsely on activated B lymphocytes.[19-22] To date, however, little is known about its distribution in lesional cutaneous tissue.

We have undertaken studies to support the concept that γ-IFN derived from dermal activated T lymphocytes is responsible for induction of keratinocyte HLA-DR expression in the relevant diseases. After immunohistochemical assessment of the relationship between keratinocyte HLA-DR expression and the dermal inflammatory infiltrate, activated T lymphocytes were quantified within the infiltrate using specific monoclonal markers. Subsequently, *in vitro* induction of keratinocyte HLA-DR expression by γ-IFN was confirmed.

METHODS

In situ detection of keratinocyte HLA-DR expression

Skin biopsies were obtained from adult patients with lichen planus (LP) ($n = 10$), discoid lupus erythematosus (DLE) ($n = 7$), cutaneous T cell lymphoma (CTCL) ($n = 7$), and allergic contact dermatitis (CD) ($n = 9$) (experimental positive nickel sulphate patch test reaction biopsied at 48 h). Specimens were snap frozen in liquid nitrogen, prior to 5 μm sections being cut on a cryostat at − 20°C. Using two monoclonal antibodies to class II MHC determinants, namely anti HLA-DR (Becton-Dickinson) and DA6/231,[24] all sections were subjected to a standard indirect immunoperoxidase reaction as previously described.[9] After fixing for 10 min in acetone the sections were dehydrated, and mounted, prior to viewing with a Leitz light microscope.

In situ identification and quantification of activated T lymphocytes

Helper T lymphocytes and activated T lymphocytes were identified using a range of monoclonal antibodies to different cell surface activation markers and T lymphocyte subset markers (Table 1). A three step peroxidase antiperoxidase (PAP) method was employed to increase the

TABLE 1. Monoclonal antibodies used to detect T lymphocyte subsets *in situ*

Antibody	Source	Cluster determinant	Cell type
IL-2R	Clonab	CD25	Activated T cells
OKT9	Ortho Diagnostics	CD2	Proliferating cells
Leu 3a	Becton-Dickinson	CD4	T helper cells
Leu 1	Becton-Dickinson	CD5	Pan T cell
Leu 2a	Becton-Dickinson	CD8	T suppressor cells

sensitivity of the assay. This assay was found to give consistently better results than a standard indirect immunohistochemical assay. Cells were quantified using an eye-piece graticule in the high power field of a Leitz light microscope. By counting cells on 5 μm sections serial to those assessed for HLA-DR it was possible to quantify the number of dermal inflammatory cells positive for Leu 3a, anti IL−2 receptor and OKT9 in areas where keratinocytes expressed HLA-DR. Positively labelled cells as a proportion of the total number of infiltrating cells could then be calculated. Subsequently, the immunophenotype of activated T lymphocytes was confirmed by double indirect immunofluorescence labelling of rhodamine labelled Leu 3a (IgG helper T cells) with fluorescein labelled Seralab anti HLA-DR (IgM).

In all studies, two controls were used; firstly inclusion of an irrelevant primary antibody and secondly, omission of the primary antibody.

In vitro induction of keratinocyte class II MHC antigen synthesis by γ-IFN

Normal human keratinocytes were cultured as previously described[23] for 6 days prior to the addition of recombinant human γ-IFN (Alpha-Therapeutic, West Germany). After incubation, keratinocytes were placed on plastic cover-slips, and the presence of HLA-DR assessed by a standard indirect immunoperoxidase reaction using the same monoclonal antibodies to HLA-DR as mentioned above.

RESULTS

In situ detection of keratinocyte class II MHC expression

Keratinocyte expression of HLA-DR was observed in all disorders studied. In each disease, keratinocytes consistently expressed HLA-DR immediately overlying areas of dense dermal infiltration, as assessed by dermal HLA-DR expression. Where the infiltrate was absent, so was keratinocyte HLA-DR expression (Fig. 1) although Langerhans cells expressing HLA-DR were observed. The intensity of staining was strongest in LP and CTCL. This may correspond to basal layer liquefaction in LP and lymphocyte epidermotropism in CTCL (see below).

In situ quantification of activated T lymphocytes

Activated T lymphocytes were consistently observed within the dermis underlying areas of positive keratinocyte HLA-DR expression. In all cases, more than 80% of infiltrating cells were helper T lymphocytes. Positive labelling with OKT9 was observed on 20% of all infiltrating cells, while 10% stained with anti IL−2 receptor antibody (Fig. 2). IL−2 receptor positive cells were observed within the epidermis in both LP and CTCL. In CD, IL−2 receptor positive cells were present within spongiotic vesicles. Double immunofluorescence labelling demonstrated that consistently more than 20% of helper T cells, as identified by Leu 3a, also expressed HLA-DR, implying activation.

In vitro induction of keratinocyte class II MHC antigen synthesis by γ-IFN

γ-IFN consistently induced expression of HLA-DR by cultured human keratinocytes.

DISCUSSION

This study has shown that HLA-DR positive keratinocytes are observed only in epidermis overlying a dermal infiltrate containing activated T lymphocytes. Furthermore, HLA-DR expression by keratinocytes is most consistent and intense in LP and CTCL where activated

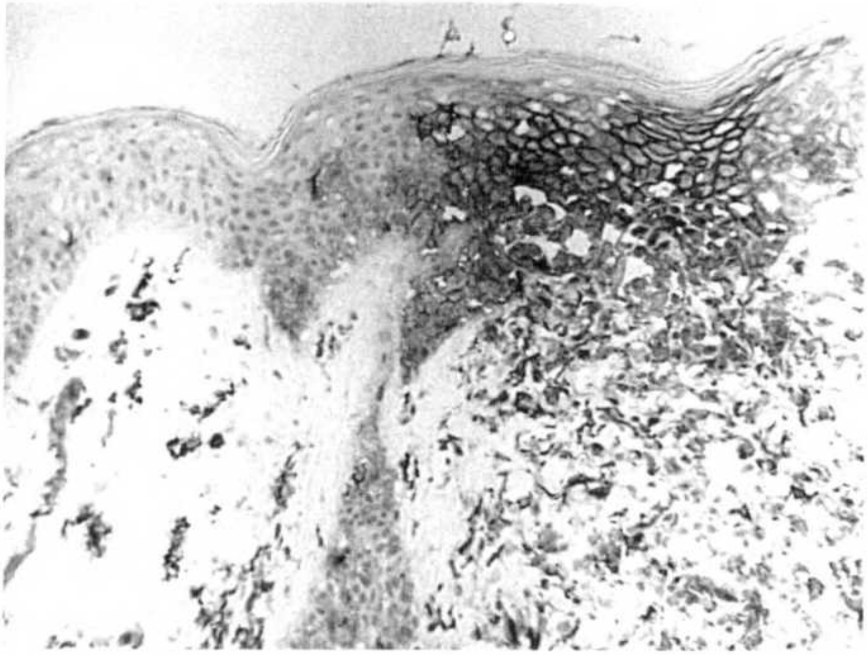

FIGURE 1. Frozen section of biopsy from lichen planus stained with anti HLA-DR. Keratinocyte membrane staining is visible on the right immediately overlying the dermal infiltrate. On the left, where there is a sparse infiltrate, the keratinocytes are not labelled. (Original × 85).

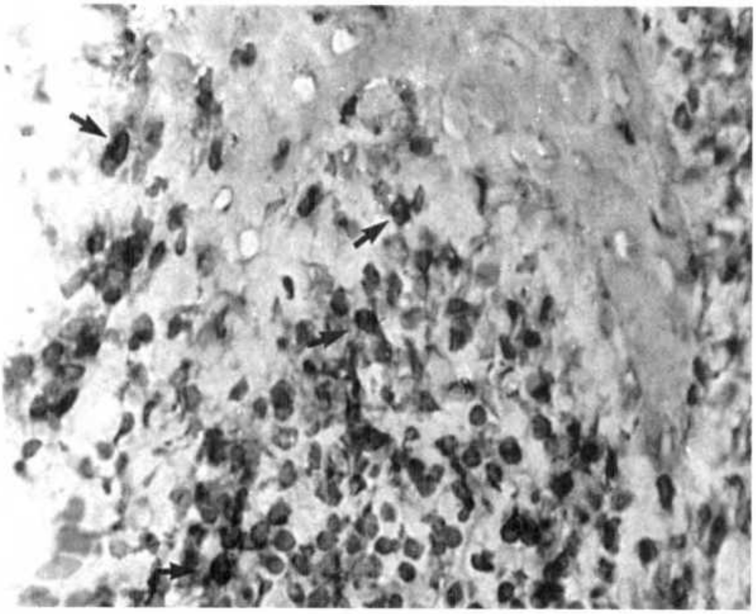

FIGURE 2. Frozen section of biopsy from lichen planus stained with antibody to interleukin 2 receptor, showing the papillary dermis. Labelled cells have dark staining membranes (arrows). (Original × 300).

T lymphocytes are present within the epidermis. This evidence strongly suggests that, in the relevant diseases, it is γ-IFN derived from dermal activated T lymphocytes that induces keratinocyte HLA-DR expression.

There is increasing evidence that in the diseases characterized by keratinocyte HLA-DR expression, keratinocytes synthesise HLA-DR and that this is induced by γ-IFN. Volc-Platzer *et al.*[25] have demonstrated *in vivo* the presence of the HLA-DR invariant chain in the cytoplasm of keratinocytes in LP and CTCL, suggesting synthesis rather than passive acquisition of shed surface antigens from neighbouring Langerhans cells. It has also been shown that, *in vitro*, keratinocytes can be induced to express HLA-DR by incubation with γ-IFN,[14,15] a result we have been able to confirm.

The source of γ-IFN has been presumed to be activated T lymphocytes within the dermal inflammatory infiltrate. We have demonstrated firstly that consistently more than 20% of infiltrating cells are activated, as determined by two different cell markers—OKT9 and double labelling with Leu 3a and anti HLA-DR. The number of IL – 2 receptor positive cells was considerably lower. There are two possible explanations. Firstly, the sensitivity of the assay may have been too low to detect regularly the IL – 2 receptor. Secondly, it has been shown that activated T lymphocytes only express IL – 2 receptor at specific stages of the cell cycle.[26] Thus, they may be found to express transferrin receptor and HLA-DR in the absence of IL – 2 receptor. The close spatial relationship of activated T lymphocytes to keratinocytes expressing HLA-DR, strongly suggests that activated T lymphocyte-derived γ-IFN, a soluble mediator, is responsible for the induction of keratinocyte HLA-DR expression. From our results, we cannot state the number or density of dermal activated T lymphocytes necessary to induce keratinocyte HLA-DR synthesis. However, lack of activation may explain why in other dermatoses characterized by a lymphocytic infiltrate, keratinocytes do not express HLA-DR, as occurs for example in atopic dermatitis.[10]

γ-IFN induces HLA-DR synthesis by activating the HLA-DR gene on chromosome 6, which in the keratinocyte is usually inactive. Messenger RNA (mRNA) is then transcribed which regulates cytoplasmic HLA-DR biosynthesis. Molecular probes now exist to HLA-DR mRNA making its detection possible within cells. *In situ* detection of keratinocyte mRNA for HLA-DR in the relevant diseases would provide proof of keratinocyte synthesis of HLA-DR.

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Intestinal mucosal mast cells: enumeration in urticaria pigmentosa and systemic mastocytosis

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SUMMARY

Endoscopic gastrointestinal mucosal biopsy specimens from one patient with systemic mastocytosis and five with urticaria pigmentosa (UP) were fixed with Carnoy's reagent and then stained for chloracetate esterase. The mast cell population densities were enumerated in the mucosa using cursor planimetry. Compared with controls, mast cell counts were increased in gastric and duodenal but not sigmoid mucosae. On a histological basis, systemic involvement would appear commoner in urticaria pigmentosa than is generally expected. Gastrointestinal symptoms did not relate to elevated mucosal mast cell counts.

Mastocytosis is characterized by excessive infiltration of tissues by mast cells. The mast cell contains a range of potent mediators which may affect organs that are not infiltrated by the mast cell process.[1] These effects include flushing, wheezing, diarrhoea or syncope, which may be induced by systemic exposure to mast cell degranulating agents or alcohol.[2,3]

When the involvement appears clinically to be confined to the skin, it is termed urticaria pigmentosa (UP). When other organs such as the gastrointestinal tract, spleen, lymph nodes or bone are involved, the term systemic mastocytosis is used. At least 10% of patients with cutaneous mastocytosis are believed to have the systemic form.[4] Gastrointestinal symptoms have been reported in 23% of mastocytosis patients, whether there is systemic involvement or not,[5] and include nausea, vomiting, diarrhoea, epigastric discomfort, colic, peptic ulceration, and steatorrhoea.[6–8]

In the rat there is a distinct sub-population of mast cells, mucosal mast cells (MMC) in the gastrointestinal tract which differ from connective tissue mast cells (CTMC) in staining and fixation characteristics.[9] However, labelling of gastrointestinal mucosal mast cells for histometric purposes requires fixation with Carnoy's reagent or basic lead acetate,[10] because with formaldehyde fixation the counts obtained are a marked underestimate.

An increase in the gastrointestinal mast cell population has been described in mastocytosis by some workers[7,11] but not others.[12] However, in these studies, details of fixation and counting

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techniques were not given. An expanded mucosal mast cell population has also been noted in untreated coeliac disease[13] and *Trichinella* bowel infestation.[14]

The present study was, therefore, undertaken to evaluate the gastrointestinal mucosal mast cell population in a group of mastocytosis patients, and to relate these results to gastrointestinal symptoms.

METHODS

Patients

All patients gave written informed consent, and the study was approved by the St. Thomas' and St. John's Hospital Ethical Committees. Gastric, duodenal and rectal biopsies were obtained by standard endoscopy techniques from six subjects with mastocytosis (four male; two female; aged 27–63 years). All had extensive maculopapular cutaneous mast cell lesions histologically confirmed, and one also had clinical and experimental evidence of systemic mastocytosis (Table 1). Four had had intermittent gastrointestinal symptoms for at least a year, with epigastric pain, nausea or diarrhoea. One patient (Patient 6) had histological evidence of systemic mastocytosis with bone and marrow involvement, and while he gave a history of headaches and bone pain, other recognized systemic symptoms of alcohol or aspirin induced flushing, wheezing or syncope were absent. None had polycythaemia.

A control group of 18 subjects (nine female; aged 26–70 years) undergoing routine endoscopic investigation of abdominal discomfort, vomiting, diarrhoea or rectal bleeding were also studied (Table 2). Ten with normal findings at sigmoidoscopy had paired rectal biopsies and eleven with normal findings at gastroduodenoscopy underwent paired gastric and duodenal biopsies. All subjects had negative stool examinations for ova and parasites.

Histological examination

Staining procedure. Following immediate immersion in Carnoy's fixative (16 ml absolute ethanol: 30 ml chloroform: 10 ml glacial acetic acid) for at least 2 h, blocks were paraffin embedded, and 3–5 sections stained for chloracetate esterase.[15] This method stains the mast cells brilliant red against a pale yellow background[16] and was chosen in preference to toluidine blue for clear identification.

TABLE 1. Clinical details of mastocytosis patients studied

Subject no.	Sex	Age (years)	Macular cutaneous lesions	Duration of lesions (years)	Gastrointestinal symptoms	Bone marrow involvement	Skeletal involvement
1	M	63	+	5	Heartburn	Absent	NT
2	F	30	+	16	Nausea Epigastric pain	Absent	Absent
3	F	27	+	3	—	NT	NT
4	M	34	+	10	Intermittent diarrhoea	Absent	Absent
5	M	59	+	15	—	NT	Absent
6	M	47	+ +	2	Epigastic pain	Present	Present

+ Generalized; + + severe generalized; NT = Not tested; — = no symptoms
Other than Patient 6 none experienced systemic symptoms of mastocytosis

TABLE 2. Clinical details of control subjects

Subject no.	Sex	Age (years)	Gastrointestinal symptoms	Endoscopy technique
1	F	62	Abdominal pain	G
2	M	70	Abdominal pain	G
3	M	32	Diarrhoea	S
4	F	28	Abdominal pain	G
5	M	40	Abdominal pain	G
6	M	32	Vomiting	G
7	F	26	Diarrhoea	S
8	F	53	Abdominal pain	G
9	F	66	Diarrhoea	S
10	F	59	Abdominal pain	G
11	M	60	Colonic polyps	S
12	F	62	Rectal bleeding	S
13	F	38	Diarrhoea	S
14	M	56	Abdominal pain	G
15	M	62	Abdominal pain	G
16	M	71	Colonic polyps	S
17	F	65	Abdominal pain	G
18	M	29	Heartburn	G

S = sigmoidoscopy only
G = gastroduodenoscopy

Counting of mast cells. Sections were recut when necessary to obtain well oriented villi cut perpendicularly. Slides were coded and all measurements were made blind. The mucosal area of each section was defined as the tissue area of the epithelium and lamina propria above the muscularis mucosa. Sub-mucosal mast cells, defined as those lying beneath the muscularis mucosa, were not counted. The area of each section to be counted was measured by cursor planimetry using a semi-automated apparatus (Imagan Graphic Information Systems, Blairgowrie, Scotland). Mast cells within this area were counted only if multiple granules and mononuclear morphology were evident. Positively staining cells with polysegmented nuclei, representing neutrophils, were excluded. Eosinophils, plasma cells, lymphocytes, and fibroblasts remained unstained with this technique.

Statistical analysis

As the results were not normally distributed, comparisons were made using the Wilcoxon rank sum test.

RESULTS

The densities of mucosal mast cells in mastocytosis patients were significantly greater than those of the control group in specimens of gastric mucosa ($P < 0{\cdot}01$) and duodenal mucosa ($P < 0{\cdot}01$). No significant difference was found between the rectal biopsy specimens in the two groups (Fig. 1). Section areas counted ranged from 1·2 to 4·4 mm^2. However, as there was a wide scatter of results in the mastocytosis group, there was overlap between mast cell densities in the three biopsy groups.

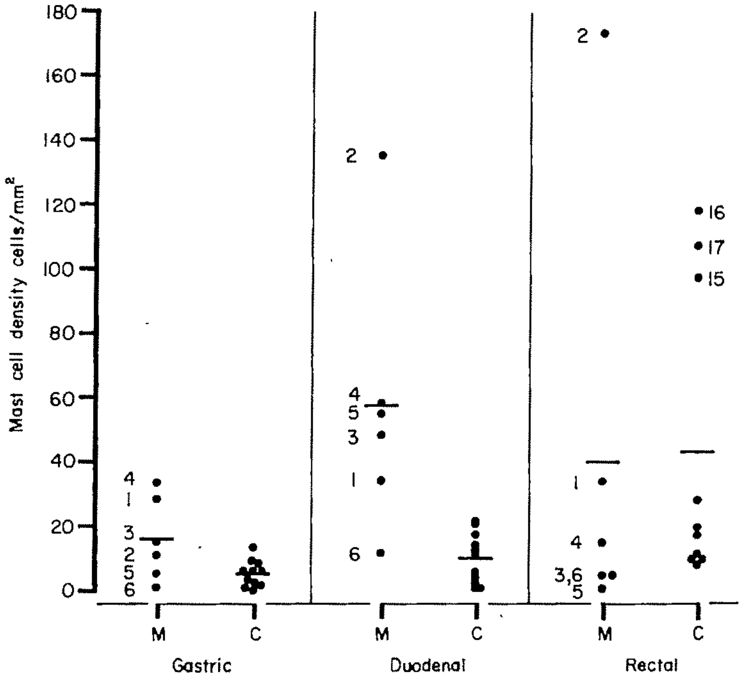

FIGURE 1. Mast cell densities in gastric, duodenal and rectal mucosal biopsy specimens from mastocytosis patients (M) and control subjects (C). Bars represent means. Numbers are case numbers as listed in Tables 1 and 2.

There was no clear relationship between mast cell counts and gastrointestinal symptoms. Thus, mastocytosis Patient 2 had high mast cell counts in both duodenal and rectal biopsies and this related well to gastrointestinal symptoms, but Patient 6, who had systemic mastocytosis and chronic gastrointestinal disturbance, showed the lowest mast cell count of the entire group. Within the mucosal area of each section, mast cells were evenly distributed with no clumping.

DISCUSSION

Counting of mast cells in the mucosa of the gastrointestinal tract has not been previously described in mastocytosis. The previous use of formalin fixative rather than Carnoy's fixative or basic lead acetate has probably greatly reduced the cells counted through failing to fix all mucosal mast cells. Chloracetate esterase labelling of Carnoy's fixed mucosal specimens produced a consistent vivid red staining of mucosal mast cells with a clarity not consistently achieved with toluidine blue.

Duodenal and gastric mucosal mast cell density was higher in the mastocytosis group than in the controls, but there was no relationship between these counts and gastrointestinal symptoms. Thus, the patient with systemic mastocytosis and chronic gastrointestinal symptoms (Patient 6) had the lowest mucosal mast cell counts of the group, and conversely two urticaria pigmentosa patients (Patients 3 and 5), without gastrointestinal symptoms, had high mucosal mast cell counts. While it is possible that the gastrointestinal symptoms in Patient 6 may be due to mediator release from mast cells elsewhere, it is also possible that, as in the skin, gastrointestinal mast cell involvement is patchy and, therefore, can be missed by paired endoscopic biopsy specimens. There was, however, no obvious variation in mast cell density within the sections. Mast cells in gastrointestinal mucosa are a sub-population that are tryptase positive, but chymase negative. However, skin and gastrointestinal submucosal mast cells are both chymase and tryptase positive.[17] Further studies should include enumeration of both the submucosal and mucosal mast cells. Biopsies should contain adequate submucosa, frequently absent in our study material.

Although it is ethically not possible to study completely healthy volunteers, the mast cell population density in controls investigated for gastrointestinal symptoms overlapped with values in the mastocytosis group, so no clear distinction can be made between mast cell population density values in gastrointestinal mucosal samples from control individuals and mastocytosis patients. Nevertheless, systemic mastocytosis with skin and histological gastrointestinal involvement may be more common than expected. It is possible that ingested mast cell degranulators act upon an expanded mucosal mast cell population and so produce systemic, or in some cases gastrointestinal, effects. In mastocytosis, treatment with disodium cromoglycate[18] as well as the H_1 and H_2 receptor antagonists may be beneficial[19]. We found this to be so in our patients.

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Mast cells and IgE in intestinal mucosa in adult atopic dermatitis patients

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SUMMARY

Duodenal biopsies from 29 adult atopic dermatitis (AD) patients with multiple positive skin prick test reactions were examined and the results compared with biopsies from 13 non-atopic controls. The duodenal mucosa showed mild inflammatory changes in six out of the 29 patients, but was normal in all the controls. Numerous anti-IgE positive cells, increasing with the severity of AD, were found in the duodenal mucosa in 25 of the 29 AD patients compared with few sporadic positive cells seen in only two out of 13 controls ($P < 0.001$). The total serum IgE level showed a significant positive correlation with the number of anti-IgE stained cells in the mucosa ($P < 0.05$). No significant differences were found in the total number of toluidine blue stained cells or cells immunoreactive for histamine between patients and controls. However, AD patients who had high numbers of anti-IgE positive cells often had decreased numbers of histamine immunoreactive cells in the mucosa suggesting mast cell degranulation. These findings provide further evidence that also in adult AD patients the gastrointestinal tract may serve as a portal of entry for allergens which may lead to exacerbation of AD.

Food allergy has been widely accepted as one of the causes of childhood atopic dermatitis (AD), but the significance of ingested allergens in the development of adult AD is debated.[1,2] In certain cases of AD, exacerbations of the disease are connected with positive skin prick tests (SPT) with food products.[3] In the gastrointestinal (GI) tract occasional permeability changes have been reported in both children and adults with AD.[4] Morphologically, only minor local changes, for example partial villous atrophy, have been observed in the mucosa of the small intestine, and these appear only in some AD patients.[5–7] A relatively frequent finding has been an increased number of IgE-immunoreactive cells in the lamina propria of the small intestine in AD patients.[5,7,8] Furthermore, local IgE synthesis has been proposed, on the basis of the detection of allergen-specific IgE in intestinal secretions.[9,10] During food provocation studies Reimann *et al.*[11] found oedema and petechial bleeding in the gastrointestinal mucosa with a

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simultaneous increase in plasma histamine levels, suggesting that the allergen reacted with the intestinal mast cells.

Interestingly, IgE receptors have been found on cell types other than mast cells, for example macrophages, platelets, B and T lymphocytes[12] and Langerhans cells.[13] Thus, IgE could be involved also in the other types of immunological reaction. In animal studies, Askenase and Van Loveren[14] found that mast cells were essential to the development of cell mediated contact allergic reactions. Reitamo *et al.*[15] demonstrated with patch testing that AD patients also showed delayed type reactions to inhalant allergens. On the other hand, the gastrointestinal tract might serve as a portal of entry not only for food allergens, but also for airborne allergens, since airborne material can be deposited in the nasopharynx from where it can pass easily into the GI tract.[16] It is tempting to think that ingested and intraluminal allergens could initiate certain immediate and delayed type reactions in AD by specific binding to IgE on target cells in the GI tract, leading to exacerbation of symptoms.

The correlation of the GI findings in AD patients with their clinical status remains unconfirmed. We have studied, therefore, the numbers of mast cells and the distribution of IgE and histamine in the duodenal mucosa of adult AD patients and correlated these findings with SPT results and the patients' clinical symptoms.

METHODS

Twenty nine patients with AD (10 male, 19 female; age range 19–45 years, mean 35 years) were included in the study. Their symptoms varied from mild dermatitis, Group A (1 to 3 sites affected, only occasional need for grade I corticosteroids) to moderate, Group B (2–4 sites affected, need for grade II corticosteroids and antihistamines almost daily) and severe, Group C (four or more sites affected, episodes of vesiculation, need for grade III corticosteroids and antihistamines). Nine patients belonged to Group A, 13 to Group B and 7 to Group C. Thirteen control patients (six male, seven female, age range 2–70 years, mean 31 years) without atopic symptoms were studied at the same time. These patients had suffered from GI problems and had, therefore, been sent for gastroscopy which had given negative findings.

The AD patients were subjected to SPT with 30 allergens including pollens, moulds, yeasts, epithelia, house dust mite (ALK-Copenhagen, Denmark) and food products including milk, egg, cereals, vegetables and spices. The reactions were compared with those to histamine chloride (3 mg/ml) and diluent, and they were interpreted as recommended.[17] The total serum IgE concentration was determined by PRIST (Pharmacia, Uppsala, Sweden).

The AD patients and controls were subjected to oesophagogastro-duodenoscopy. Biopsy specimens were taken from the descending duodenum with an Olympus GIF-XQ 10 endoscope using standard forceps (Olympus FB-24 K or FB-25 K). All the biopsy specimens were placed on a fibre free card for histological evaluation. The technique was similar to that described for confirmation of the diagnosis of gluten sensitive enteropathy.[18] The biopsy specimens were fixed routinely with 4% phosphate buffered formaldehyde and embedded in paraffin. For histology, sections were stained with haematoxylin and eosin and toluidine blue (at pH 0·5 for 5 days[19]). For immunocytochemistry, we applied rabbit anti-IgE antiserum (Dakopatts A/S, Glostrup, Denmark) and rabbit anti-histamine antiserum.[20,21] Serial dilutions were tested to obtain ideal conditions and a 1:5000 dilution of anti-IgE and a 1:8000 of anti-histamine were chosen. The bound primary antibodies were demonstrated using the avidin-biotin-peroxidase method (Vectastain, Burlingame, PA, USA) and 3′,3′-diaminobenzidine to develop the colour.

A skin biopsy from a patient with urticaria pigmentosa was used as the positive control for the staining; toluidine blue, anti-IgE and anti-histamine all stained the mast cells intensely. For negative controls the primary antiserum was substituted with normal rabbit serum (Dakopatts) and in the case of anti-histamine also with immunoadsorbed serum; normal serum gave negative results and adsorption of the anti-histamine antiserum with the specific antigen abolished the immunoreactivity. The mucosal cells were quantified using a Leitz Dialux 22 light microscope (eyepiece × 12·5 with framing marks 6·5 × 9, objective × 40). In each case the number of cells in five different areas of the mucosa above the muscularis mucosae were counted giving the number of cells/five visual fields.

Student's *t*-test was used for statistical analysis.

RESULTS

Total IgE and skin prick tests

The serum total IgE concentration ranged in Group A from 20 to 7200 U/ml (mean 1650), in Group B from 32 to 13 600 U/ml (mean 1698) and in Group C from 165 to 10 500 U/ml (mean 4436). In SPT with inhalant allergens performed earlier all patients were positive. Four of seven patients in Group A and nine of 13 in Group B were SPT positive with different food products. In Group C, three of the seven patients could not be skin tested because of their widespread dermatitis; they had, however, positive IgE RAST values (Pharmacia, Uppsala, Sweden) for egg, milk and cereals. All four patients in Group C who could be subjected to SPT were positive.

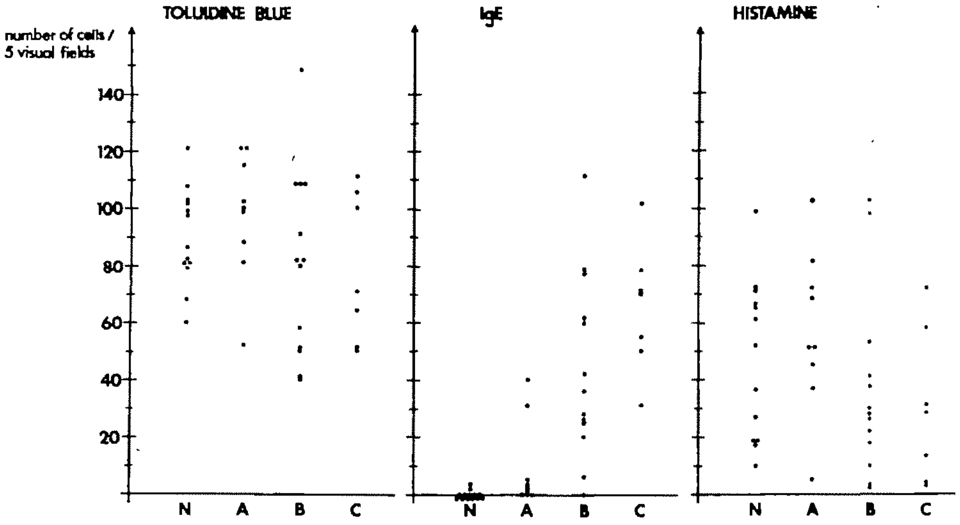

FIGURE 1. Numbers of cells stained with toluidine blue, and antisera to IgE and histamine in the duodenal mucosa of patients with mild (A), moderate (B) and severe (C) atopic dermatitis and non-atopic controls (N).

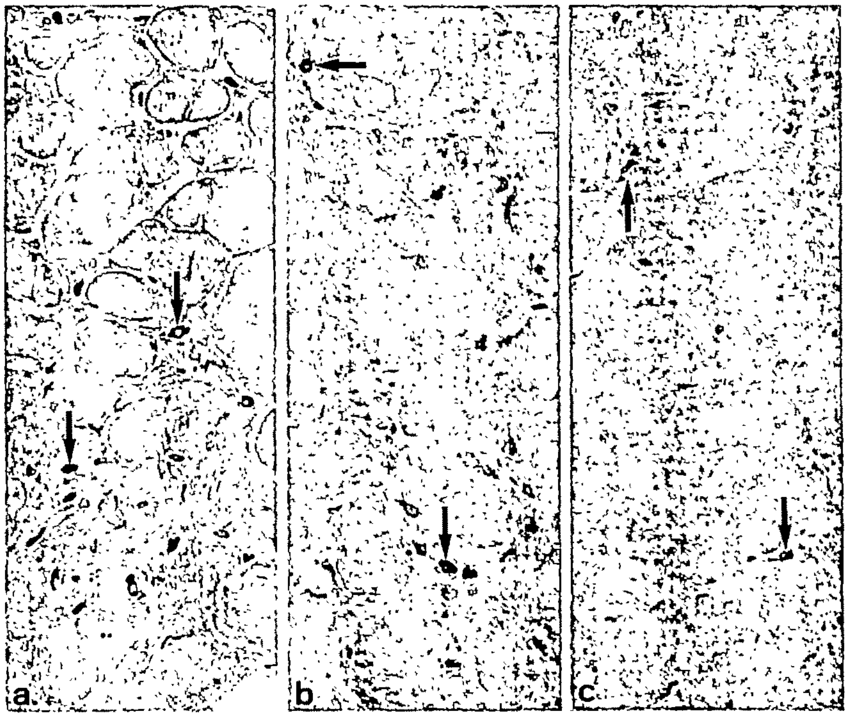

FIGURE 2. Duodenal mucosa from a patient with severe atopic dermatitis. The sections, close together in the same series, show that in regions with numerous toluidine blue positive mast cells (two marked with arrows) (a) there are also many IgE positive cells (arrows) (b), whereas the number of cells immunoreactive for histamine is low (c).

Histological changes in the duodenum

Six of the nine patients in Group A had a normal mucosa, and three had mild duodenitis. In Group B, five patients were normal and eight had mild duodenitis. In Group C, three patients were normal and four had mild duodenitis. All biopsies from the control patients showed a normal duodenal mucosa.

Toluidine blue, anti-IgE and anti-histamine staining

The numbers of positively stained cells in the duodenal mucosa are shown in Figure 1. An example of the staining pattern in a patient with severe AD is shown in Figure 2 a, b and c. The mucus on the luminal cell surface in patients in each group showed intense anti-IgE staining as did the goblet cells. In addition, we found that the number of anti-IgE positive cells in the mucosa was significantly greater in the AD patients than in the controls ($P < 0{\cdot}001$). The total serum IgE level showed a significant positive correlation with the number of mucosal anti-IgE positive cells ($P < 0{\cdot}05$) (Fig. 3), except for three of the patients who showed a few mucosal cells staining for IgE despite a normal total serum IgE level. Furthermore, there was a negative correlation between anti-histamine positive cells and anti-IgE positive cells. No significant differences in the total number of toluidine blue or anti-histamine positive cells could be found between the patients and controls.

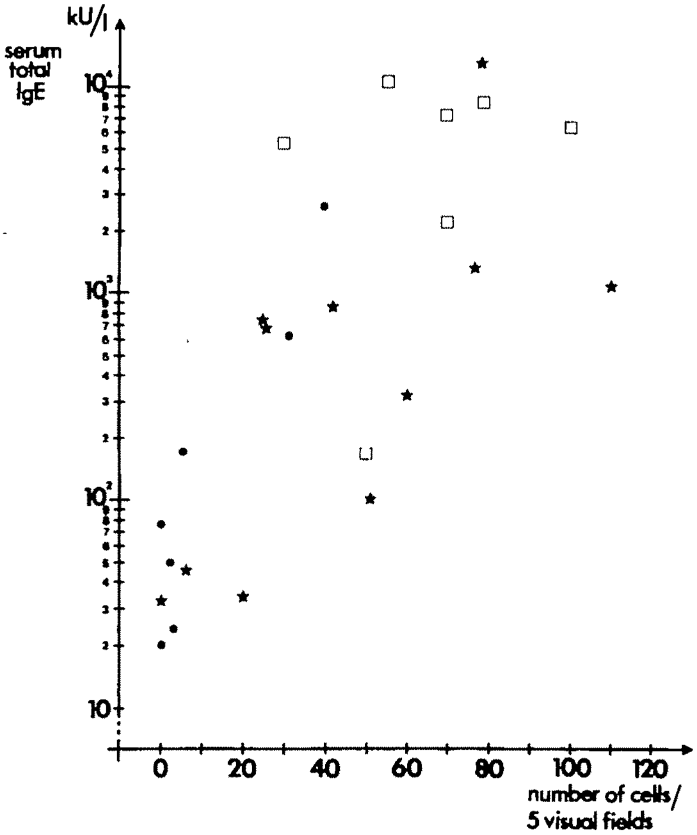

FIGURE 3. The relationship between serum total IgE and anti-IgE staining cells in the duodenal mucosa of 25 atopic dermatitis (AD) patients. ●, Mild AD; ★, moderate AD; □, severe AD.

DISCUSSION

In some previous studies, IgE positive cells were not found in the mucosa of AD patients by immunofluorescence or the direct immunoperoxidase method,[6,22,23] whereas a few cases with IgE positive cells were described by Shiner *et al.*,[8] Braathen *et al.*[5] and Rosekrans *et al.*[7] Our results demonstrate that IgE immunoreactive cells are present in considerable numbers in the mucosa of the majority of those adult AD patients who show positive skin prick test reactions or RAST values, and the number of these cells correlated well with the clinical severity of the disease and the IgE level in the blood. Furthermore, in patients with more severe AD the numbers of histamine positive mast cells decreased, suggesting degranulation of the cells in response to continuous allergen exposure.

The existence of food allergy in adult AD patients has been doubted,[2] but good evidence in favour has also been presented, for example, an increase in serum histamine levels after food provocation tests was demonstrated by Reinman *et al.*[11] Recently, Carini *et al.*[24] were able to demonstrate the appearance of complexed IgE and deterioration of symptoms after food challenge in patients with asthma, rhinitis or eczema. We regard our results as further evidence for the view that the intestinal mucosa can act as one portal of entry for the allergens since it is likely that the mucosal mast cells did react to allergens arriving via the GI tract. On the other hand, since not only food, but also considerable amounts of swallowed inhalant allergens from the nasopharynx[16] may enter the GI tract, it remains uncertain whether this intestinal reaction reflects food or inhalant allergy. Since most of our patients (increasing numbers with more severe AD) showed positive reactivity to food products in other tests we consider it most probable that food can contain allergens that induce an immune response, very probably close to their site of entry in the intestinal lymphoid tissue.

The relationship between these GI findings and the patient's skin symptoms is still a matter of speculation. AD is a multifactorial disease with various triggering mechanisms. In certain cases it could be hypothesized that the allergens entering the GI tract are absorbed and passed on to the blood circulation. The inflammatory reaction in the intestine, noted in our patients, could further increase the permeability to these allergens. In this way the allergens could become widely distributed through the body and reach the skin where they could induce a delayed immune reaction, thus worsening the atopic dermatitis. On the other hand, histamine and other inflammatory mediators liberated from the activated cells may contribute also and cause exacerbation of AD. It may also be that the circulating IgE synthesized in response to the food allergens attaches to the IgE receptors on macrophages and Langerhans cells[12,13] in the skin and triggers the delayed immune reaction after new allergen exposure. The suggested role of mast cells in the cell mediated allergic reaction[14] could also be the link between allergens and AD.

Thus, our results provided further evidence that the GI tract does serve as a portal of entry for allergens, initiating inflammatory reactions which may manifest through different pathomechanisms in the exacerbation of AD.

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A study of local immunity in psoriasis

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SUMMARY

We have studied biopsies of lesional skin with monoclonal and polyclonal antibodies to determine the nature of the infiltrate in different forms of psoriasis. In guttate psoriasis the number of Langerhans cells (LC) was not increased and CD8+ cells predominated, however, most CD4+ cells were activated. In erythrodermal psoriasis some CD1+ cells were found in the dermis although CD8+ cells predominated and were activated. In palmo-plantar psoriasis LC were found in the epidermis and superficial dermis and CD4+ cells were more numerous than CD8+ cells, some in turn expressing HLA-DR. In flexural psoriasis the LC formed clusters in the epidermis, and activated CD4+ cells predominated. In psoriasis vulgaris CD4+ cells predominated over CD8+ although the latter were activated. Our observations tend to support Valdimarsson's hypothesis on the immune basis of psoriasis, but we believe that two cycles coexist in psoriasis—one inflammatory and the other immunological—and that interaction between these leads to epidermal hyperproliferation.

Psoriasis is a chronic dermatitis of unknown aetiology. The condition is characterized by outbreaks of papules and erythemato-squamous patches; the most evident alteration is the increase in epidermal proliferation.

A number of anomalies have been detected in psoriasis related to inflammation and immunity. An increase has been observed in the content of arachidonic acid and its derivatives—PGE_2, PGF_2 and 12-HETEs—in both affected and unaffected skin.[1] Quantitative alterations in cyclic nucleotides (cAMP and cGMP) have also been found,[2] together with an increase in polyamines.[3] The squama have revealed the presence of leukotriene B_4. The β-adrenergic receptors in psoriasis have been found to be defective.[5] A drop in circulating T lymphocytes has been reported,[6] and the number of Langerhans cells in both affected and unaffected skin is lower than in non-psoriatic subjects.[7] At infiltrate level, T4 lymphocytes predominate over T8 cells, monocytes in turn constituting a minority.[8] Anti-stratum corneum antibodies are observed in healthy individuals, although their presence is much greater in psoriasis patients. The same applies to the activation of the complement C_3 fraction.[9] The neutrophils of these patients synthesize 2·4 times as much leukotriene B_4 as those of healthy individuals.[10]

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Three hypotheses have been proposed to explain the pathogenesis of psoriasis; Voorhees[11] attributes the condition to a defect in cyclo-oxygenase, where the arachidonic acid metabolites derive from the lipoxygenase pathway. According to Farber,[12] the neuropeptides are of particular importance in causing the lesions and inflammatory-immune alterations. Valdimarsson,[13] in turn, proposes the existence of an antigen capable of stimulating the Langerhans cells to cause entry of T4 lymphocytes into the epidermis, where these cells would induce an immune reaction. The latter would only recede with the entry of T8 cells.

None of these hypotheses provides a complete explanation of the pathogenesis of psoriasis. The present study was consequently designed to verify the occurrence of immune changes in psoriasis.

METHODS

Patients

Twenty-eight patients (16 males and 12 females), aged 14 to 52 years, with psoriasis, confirmed clinically and histologically, were studied. Six had guttate psoriasis, two psoriatic erythroderma, five palmo-plantar psoriasis, three flexural psoriasis and 12 psoriasis vulgaris.

Specimens

Biopsies of lesional skin were obtained from all patients using a 6 mm punch. Each biopsy was divided into two portions; one was frozen in liquid nitrogen and stored at −50°C. The other portion was fixed in buffered, isotonic 10% formaldehyde and embedded in paraffin; sections 4 μm thick were cut and stained with haematoxylin and eosion.

Cryostat sections 4 μm thick were cut from each frozen specimen. The sections were fixed at −20°C in acetone before incubation with monoclonal or polyclonal antibodies.

Monoclonal antibodies

Monoclonal antibodies used in the study were OKT3 (CD3), OKT4 (CD4), OKT8 (CD8), OKT6 (CD1) (Ortho Diagnostics, New Jersey, U.S.A.) and anti-HLA-DR (Becton Dickinson, U.S.A.).

Conjugated anti-mouse antibodies

RAM/TRIC IgG, conjugated with rhodamine and GAM/FITC IgG, conjugated with fluorescein were obtained from Nordic Immunology, The Netherlands.

Other reagents

Other reagents used were phosphate buffered saline (PBS), pH 7·4 (Bio-Merieux, France); System Avidin-Biotin (Vector, U.S.A.); Polyclonal anti-F′ab serum (Boehringer, FRG) and 3-triethyl-carbazole (Sigma, U.S.A.).

Staining techniques

Avidin-biotin technique. Endogenous peroxidase was inhibited by hydrogen peroxide, after which the sections were covered with horse serum for 15 min, washed with PBS and then incubated for 45 min with the monoclonal antibody. The sections were washed again in PBS, covered with biotinilized serum for 4 min, washed again and then avidin-biotin complex was applied. Peroxidase was developed with 3-triethyl-carbazole for 10–20 min, and the sections counterstained with Mayer's haematoxylin, and mounted in gelatine/glycerine medium.

Double labelling technique with rhodamine/fluorescein. In order to determine the different proportions of the various cell types in the infiltrate, two monoclonal antibodies were used on the same section and developed with two conjugates—one with rhodamine and the other with fluorescein. The following pairs of monoclonal antibodies were used:

Anti-T3 and anti-B; anti-T4 and anti-T8; anti-T4 and anti-HLA-DR; anti-T8 and anti-HLA-DR; anti-T6 and anti-HLA-DR.

The sections were incubated with the first monoclonal antibody for 45 min, washed with PBS, incubated with the first conjugate for 45 min and washed again in PBS. The sections were then incubated with the second antibody for 45 min and washed with PBS; the second conjugate was then applied for 45 min. Finally, after washing again in PBS, the sections were mounted in glycerol/phosphate medium.

Sections were examined under a Nikkon type Labophot microscope fitted with an epi-illumination device with a mercury vapour lamp and filters suitable for fluorescein and rhodamine.

The results were recorded as discrete predominance if one cell type represented between 50 and 75% of the total, predominance where one cell type represented 75% of the overall total, and great predominance if one population was in excess of 75% of the total, without reaching 100%.

RESULTS

The results are shown in Tables 1 and 2.

In all cases studied the histopathological diagnosis was compatible with the clinical presentation. No significant decrease in the number of epidermal Langerhans cells was observed.

TABLE 1. Distribution of CD1+, CD4+ and CD8+ cells in different types of psoriasis

	Location of cells		
Type psoriasis	CD1+	CD4+	CD8+
Guttate	Suprabasal Ep	Pd; Sd	Ep; Pd; Sd; Pv
Erythrodermal	Ep	Sd	Sd; Md
Palmo-plantar	Ep (Clusters) Sd	Ep; Pd; Sd	Sd; Md
Flexural	Ep (Clusters) Sd	Pd; Sd	Ep; Sd; Md
Vulgaris	Ep Sd (Clusters)	Ep; Pv; Pd; Sd	Sd; Md

Ep = Epidermis, Pd = Papillary dermis, Md = Mid dermis, Sd = Superficial dermis, Pv = Perivascular.

TABLE 2., Relative composition of the lymphocytic infiltrate in different types of psoriasis

Type of psoriasis	Relative numbers of cells				
	CD$_3$+, B	CD$_4$+, CD$_8$+	CD$_8$+, HLA-DR	CD$_4$+, HLA-DR	CD$_1$+, HLA-DR
Guttate	CD3 > B	CD4 < CD8	CD8 < HLA-DR	CD4 > HLA-DR	CD1 = HLA-DR
Erythrodermal	CD3 > B	CD4 = CD8	CD8 > HLA-DR	CD4 > HLA-DR	CD1 = HLA-DR
Palmo-plantar	CD3 > B	CD4 > CD8	CD8 < HLA-DR	CD4 = HLA-DR	CD1 = HLA-DR
Flexural	CD3 > B	CD4 > CD8	CD8 > HLA-DR	CD4 < HLA-DR	CD1 = HLA-DR
Vulgaris	CD3 > B	CD4 > CD8	CD8 = HLA-DR	CD4 < HLA-DR	CD1 > HLA-DR

Guttate psoriasis

No increase in the number of Langerhans cells is found although all simultaneously expressed the antigens CD1 and HLA-DR, their distribution being suprabasal (Fig. 1). A great predominance of CD8+ lymphocytes over CD4+ cells was found in the infiltrate (Fig. 2) and localized to the epidermis and superficial and papillary dermis and perivascular spaces. The number of HLA-DR+ lymphocytes was greater than the number of cells expressing CD8 antigen, which indicates that some CD4+ lymphocytes were activated. A great predominance of CD3+ over B lymphocytes was observed.

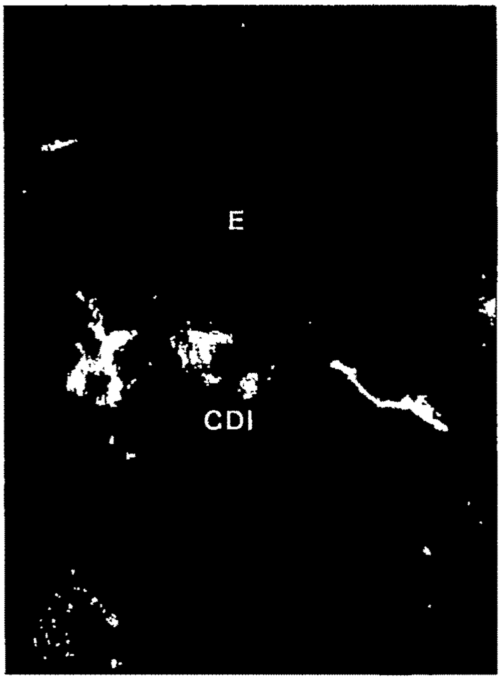

FIGURE 1. Guttate psoriasis. CD1+ cells (CD1) in the epidermis (E). Normal in number and localization. (Original × 600).

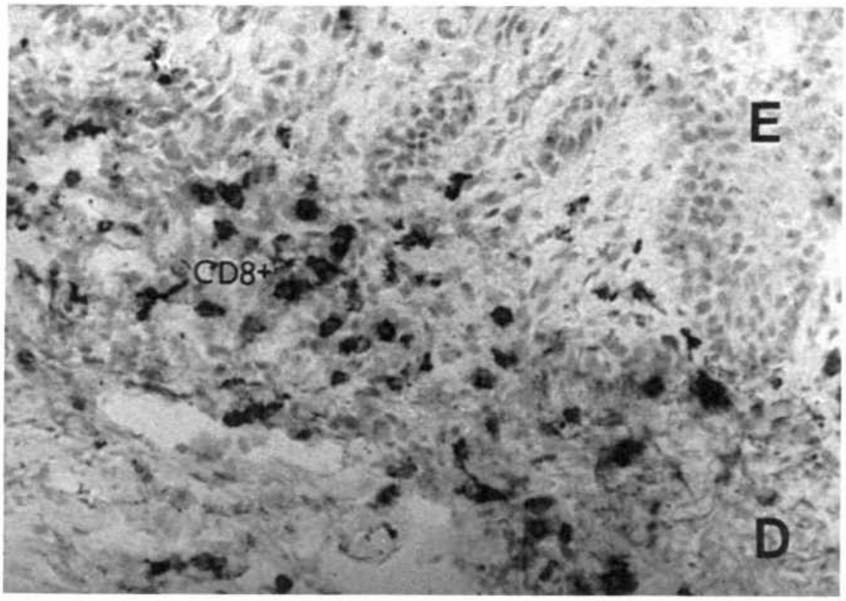

FIGURE 2. Guttate psoriasis. Distribution of increased numbers of CD8 + cells (CD8 +) in the superficial dermis. E = Epidermis, D = Dermis. (Original × 400).

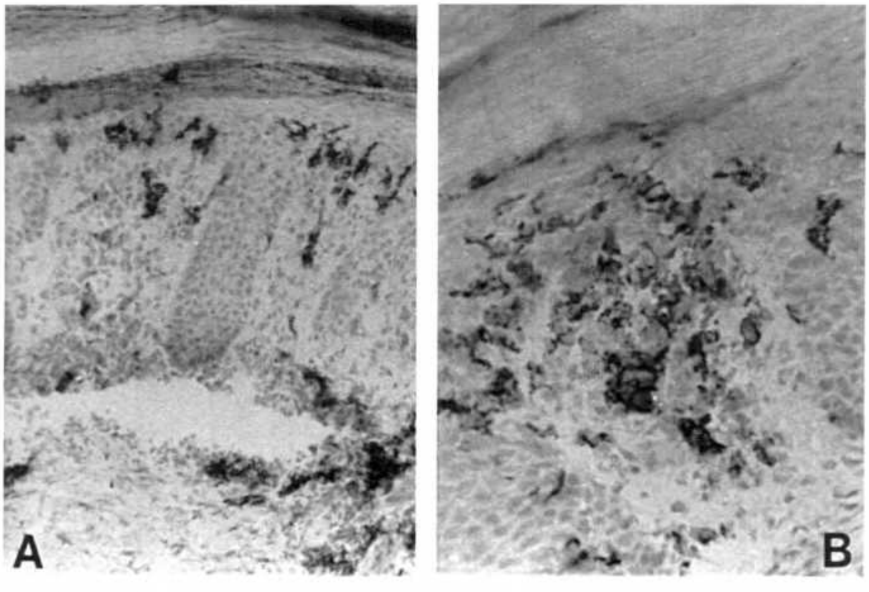

FIGURE 3. Palmo-plantar psoriasis. Distribution of the CD1 + cells in the epidermis and superficial dermis. (A) original × 200 (B) original × 400.

Erythrodermal psoriasis

The proportion of CD1 + and HLA-DR + cells in the epidermis was not increased, and the cells were located intraepidermally although a number of CD1 + cells were found within the dermis in several biopsies. The CD4 + and CD8 + cells were distributed in the superficial and mid dermis. In spite of the predominance of CD8 + cells, the number of lymphocytes expressing HLA-DR antigen was smaller than the number of CD4 + and CD8 + cells combined. The predominance of the CD3 + cells over B cells was slight.

Palmo-plantar psoriasis

The Langerhans cells were situated in the epidermis and superficial dermis (Fig. 3 a and b), and some epidermal dendritic cells were also present; no variation in the number of Langerhans cells expressing CD1 and HLA-DR was found; there was no increase in number, and no predominance of one population over the other. At dermal level the number of lymphocytes expressing T4 antigen was greater than the number of T suppressors (T_8 +), the former being mostly activated, in contrast with the CD8 + cells and some HLA-DR + lymphocytes. Some CD4 + cells were located in the epidermis, (Fig. 4 a and b). There was a great predominance of CD3 + cells over B cells.

Flexural psoriasis

A number of CD1 + cells were detected in the basal layer, epidermis and superficial and papillary dermis; at epidermal level some dendritic cells formed clusters and simultaneously expressed both CD1 and HLA-DR antigens. The CD4 + and CD8 + lymphocytes were distributed in the superficial and mid dermis, and perivascular spaces; a few CD8 + cells were found in the epidermis. The CD4 + cells were mostly HLA-DR +, and predominated over CD8 + cells; a few of the latter expressed the HLA-DR antigen. The CD3 + cells greatly predominated over B lymphocytes.

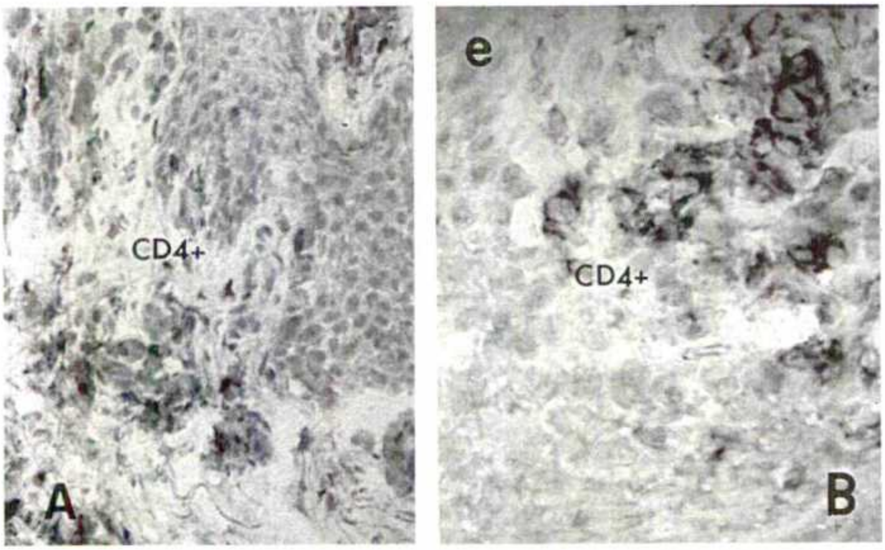

FIGURE 4. Palmoplantar psoriasis. (A) CD4 + cells (CD4 +) in the papillary dermis. (Original × 400) (B) CD4 + cells (CD4) in the epidermis. e = Epidermis. (Original × 600).

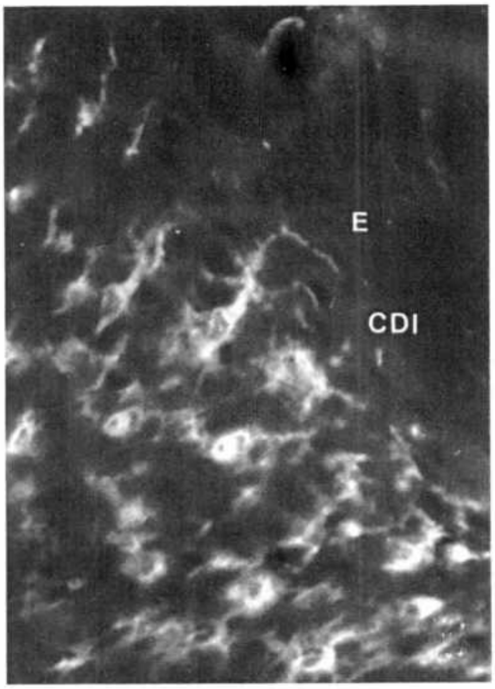

FIGURE 5. Psoriasis vulgaris. CD1 + cells (CD1) in the epidermis. Increased numbers are present in all epidermal and suprabasal layers. E = Epidermis. (Original × 600).

Psoriasis vulgaris
A predominance of CD1 + cells was found in the epidermis (Fig. 5) in comparison with the HLA-DR + population, although most sections showed CD1 + elements at the basal level or within the papillary dermis. CD4 + lymphocytes predominated over CD8 + lymphocytes, and the latter were all activated in contrast with the CD4 + cells where some cells were HLA-DR +. Some CD4 + cells were found in the epidermis. The B lymphocytes were in a minority compared with CD3 + cells.

In none of the biopsies were HLA-DR antigens expressed by the keratinocytes in the lesional areas.

DISCUSSION

The two fundamental types of reaction, immune and inflammatory, both appear in this pathology. The activation of T4 and T8 cells is accompanied by their surface expression of the class II histocompatibility antigen (HLA-DR); they may thus be easily detected by specific monoclonal antibodies (CD4, CD8 and HLA-DR). This cell activation appears to play an important role in the pathogenesis of psoriasis, according to Valdimarsson.[13]

On analysing the results it can be seen that the CD1 + cells were present in normal numbers, although they occasionally formed accumulations in the lower layers of the epidermis. In all biopsies an extensive predominance of T3 lymphocytes over B cells was found; consequently, the nature of the infiltrate points to a delayed-type immune response. Earlier studies have come to the same conclusion[14] on detecting Langerhans cells within the epidermis.

The results with guttate psoriasis indicate that a number of helper T lymphocytes are also activated, which is in agreement with Valdimarsson,[13] in the presence of a larger number of suppressor cells, these lesions should be regressing.

In erythrodermal psoriasis the finding of Langerhans cells in the dermis points to much immune activity in the lesion. In relation to the infiltrate, it would indicate an equilibrium in the dynamics of the immune response in the individual plaque lesion, with a sustained low level of activity.

The infiltrates in palmo-plantar psoriasis indicate that helper cell function exceeds that of the suppressor cells, thus keeping the lesions active.

In the case of flexural psoriasis, in spite of a greater presence of CD4+ lymphocytes, activity as indicated by the HLA-DR antigens was greater among the suppressor cells. This points to the initiation of remission or, alternatively, to the action of some external, as yet undetermined influence on the plaques, e.g. some infectious process, or the application of pharmacological or other agents. Such agents may not contain active substances, but may result in an increase in CD4+ cells and in the presence of Langerhans cells within the dermis.

In psoriasis vulgaris the results point to an increase in Langerhans cell activity in comparison to the rest of the infiltrate. In spite of a predominance of T4 lymphocytes in the infiltrate, T8 activity was greater (mostly activated cells). The presence of Langerhans cells in the basal cell layer and upper dermis contrasts with the predominantly suppressor cells infiltrate; some infectious, irritant or other element could perhaps be responsible for this.

These results, analysed from the viewpoint of Valdimarsson's hypothesis,[13] partly satisfy the latter—although many obscure points remain. It does not explain the reason for the inflammatory reaction, and fails to clarify the biochemical changes taking place within the skin. In spite of hyperproliferation, psoriasis exhibits a primary differentiation and equilibrium between basal cell production and cell loss in the horny layer. The inflammatory reaction with a polymorphonuclear infiltrate is of importance in view of the interactions which can occur between the immune and inflammatory responses.

The hypotheses put forward by Farber[12] and Voorhees[11] are the counterpart to the immune concept proposed by Valdimarsson,[13] the inflammatory reaction constitutes the central process in the first two hypotheses, and hyperproliferation is seen as a consequence of mechanisms involving inflammatory mediators, enzymes and lymphokines.

Our observations tend to support the ideas of Valdimarsson; it could be suggested that the Langerhans cells in certain individuals constantly stimulate the T4 population, as a consequence of some genetically determined alteration of the cells, through viral mediation, or as a result of distant infectious foci. Sunlight improves psoriasis and leads to a drop in the number of Langerhans cells.[15] The cells described by Granstein[16] could also be of importance in the pathogenesis of psoriasis. Cyclosporin A has been used successfully in the treatment of this disease,[17] and its action centres on decreasing T cell activity.

Taking these data into account, along with the aforementioned hypotheses, it may be proposed that a consequence of this initial alteration would be an inflammatory response with production of leukotrienes and HETEs, which in turn exert a modulating influence on the immune response.[10] Among the consequences would be a chemotactic effect on the polymorphonuclear cells, which would, therefore, not release enzymes or trigger epidermal cytotoxicity.

Although further studies are required, we believe that two cycles co-exist in psorasis—one inflammatory and the other immune—and that the interaction between them leads to epidermal hyperproliferation.

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Skeletal hyperostosis and extraosseous calcification in patients receiving long-term etretinate (Tigason)

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SUMMARY

In an ongoing study of patients on long-term etretinate (Tigason) therapy, 13 patients with a congenital or inherited disorder of keratinization and 10 patients with psoriasis were examined to investigate the incidence of, and the factors associated with, skeletal hyperostosis. Skeletal scintigraphy, plain radiographs, haematological and biochemical analyses were performed. Using all criteria, 7 of 13 patients with a congenital or inherited disorder of keratinization showed evidence of hyperostosis. No single investigation was able to detect all these cases; in particular, skeletal scintigraphy was positive in only nine of the 13 patients who showed hyperostosis. Eleven of the 13 patients with hyperostosis gave a history of musculoskeletal symptoms compared with three of the 10 patients without hyperostosis. There was no clear association with total dose or duration of treatment. Serum chemistry and haematological studies were normal. In two patients the 24-h urinary calcium excretion was significantly elevated, an abnormality which has not been described previously. Annual lateral thoracic spine radiographs with additional views of symptomatic areas are recommended for patients on long-term etretinate therapy.

The synthetic analogues of vitamin A (retinoids) are widely used in the treatment of skin disorders, but are recognized as having various side-effects, particularly with chronic use. These include skeletal changes, with bone spurring and ossification of ligaments, mimicking diffuse idiopathic skeletal hyperostosis (DISH). There are reports of this as a complication of etretinate (Tigason®) therapy[1–5] although many studies include other retinoids, particularly isotretinoin (Roaccutane® and Accutane®).[6–14] Some controlled series refute an association between retinoids and hyperostosis.[9,15] There are conflicting reports regarding the relationship between total dose and skeletal effects.[6,16] The incidence of these effects is not clear nor is there agreement on the best technique for monitoring those patients on long-term therapy. The present study intended to address these questions.

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The studies of etretinate alone have had treatment groups which included patients with psoriasis[3] which can cause similar bone changes in its own right. It, therefore, might be difficult to differentiate between hyperostosis due to the underlying disease and any resulting from treatment. In an attempt to assess the incidence of skeletal changes due to etretinate alone we decided to investigate patients with congenital or inherited disorders of keratinization (DOK). A group of patients with psoriasis was also examined, selected by the same criteria.

Scintigraphy has been reported as detecting early periosteal changes due to hypervitaminosis A before there is plain radiographic evidence.[17] A study using scintigraphy to detect bone changes during long-term etretinate therapy showed no abnormality.[18] The assessment of our patients included scintigraphy as well as plain radiography to try to find a sensitive, early indicator of retinoid toxicity.

One paper has described a single case of hypercalcaemia associated with isotretinoin administration.[19] To date, to our knowledge, there have been no reports of altered calcium metabolism due to etretinate therapy, although liver function may be impaired.

METHODS

Permission for the study was obtained from the Oxford Regional Ethics Committee. A licensee for the use of radioactive substances (D.J.W.) obtained approval for the project from the Administration of Radioactive Substances Advisory Committee. Informed consent was obtained from each patient.

Male or female patients aged 18 years or more entered the trial. The patients were currently receiving long-term continuous etretinate therapy for a disorder of keratinization and had been receiving such therapy continuously for a minimum period of 3 years excluding short periods off treatment. Patients were excluded if they required regular radiographs for the assessment of a concomitant disease.

Thirteen patients were recruited through U.K. consultant dermatologists and each attended for 1 day for full radiological assessment and laboratory screening at the John Radcliffe Hospital in Oxford. Ten patients with psoriasis were studied in the same way. Patient details are given in Table 1.

Details of the clinical history, including the condition for which etretinate was prescribed, were supplied by the referring consultant. Any previous treatment with vitamin A, doses and the duration of treatment were noted. The past and current doses and duration of etretinate were documented along with any adverse effects and efficacy of therapy. Previous radiographic evidence of skeletal hyperostosis or extraosseous calcification before and during etretinate therapy were noted as well as any concomitant disorders and their treatment.

Radiological assessment commenced with a lateral film of the thoracic spine in all patients. Up to four additional films were permitted in patients with significant musculoskeletal symptoms or positive scintigraphy.

A sample of fasting blood was taken and via the same needle 500 MBq technecium HMDP (hydroxy methyl diphosphonate) was injected. After 2–3 h images of the skeleton were obtained using an IGE Maxicamera 61. Laboratory data on each patient included a full blood count, differential white count, platelets, erythrocyte sedimentation rate, liver enzymes, alkaline phosphatase, serum electrolytes, and fasting serum triglycerides and cholesterol. Twenty-four hour urinary calcium was measured from a sample collected by the patient before attendance.

The images obtained by bone scintigraphy were assessed separately by two radiologists and classified as abnormal, borderline or normal. Those considered borderline were reassessed by

TABLE I. Details of patients who took part in the study

				Etretinate				Hyperostosis				
Patient no.	Age (years)	Sex	Diagnosis	Duration (months)	Total dose (mg)	Mean dose (mg/kg/day)	Activity on bone scan	Spine X-ray	Other X-ray	Area affected	History of musculoskeletal symptoms	Urinary Ca⁺⁺ (mmol/24 h)
1	45	F	Darier's disease	57	22,350	0·23	–	–	–	...	+	5·1
2	49	M	Darier's disease	52	86,575	0·62	–	+	–	...	+	2·0
3	49	F	Darier's disease	42	47,925	0·51	–	–	–	...	–	4·2
4	53	F	Darier's disease	42	60,025	0·67	–	+	–	...	+	5·5
5	48	M	Darier's disease	75	139,940	0·81	+	–	–	...	+	1·4
6	21	M	Darier's disease	30	17,400	0·27	+	–	+	Rib	+	3·5
7	38	M	Congenital pityriasis rubra pilaris	68	101,813	0·91	+	+	+	Knees	+	3·4
8	40	F	Ichthyosis	21	5,250	0·10	–	–	–	...	–	4·8
9	25	M	Ichthyosis	40	60,000	0·65	–	–	–	...	–	3·6
10	30	M	Lamellar ichthyosis	68	100,385	0·69	+	–	+	Knees	+	0·3
11	18	M	Non bullous ichthyosiform erythroderma	40	30,980	0·34	–	–	–	...	–	5·3
12	23	F	Erythrokeratoderma variabilis	72	110,000	1·04	–	–	–	...	–	3·0
13	28	F	Erythrokeratoderma variabilis	25	16,050	0·38	–	+	–	...	–	–
14	52	M	Psoriasis	31	13,995	0·17	+	+	+	Knees	+	0·7
15	60	M	Psoriasis	44	23,025	0·23	+	+	–	...	+	2·0
16	52	M	Psoriasis	48	21,420	0·21	+	+	–	...	–	3·4
17	47	M	Psoriasis	75	94,350	0·52	+	–	+	Forearm, hips, knees	+	4·3
18	57	M	Psoriasis	39	57,125	0·83	–	–	–	...	–	2·3
19	20	M	Psoriasis	25	19,000	0·30	–	–	–	...	+	11·2
20	51	F	Psoriasis	50	60,000	0·50	+	+	+	Knees	+	9·5
21	57	M	Psoriasis	21	22,383	0·47	–	–	–	...	+	3·1
22	35	F	Psoriasis	50	36,000	0·45	–	+	–	...	+	2·0
23	58	F	Psoriasis	41	43,275	0·42	–	–	–	...	–	5·0

the radiologists working together and reclassified as abnormal or normal. They also noted whether the scintigraphic changes were due to hyperostosis or another cause, such as osteoarthrosis. Those considered to be due to other causes were classified as normal for the purposes of the present study.

To assess the accuracy of the radiologists' interpretation of plain films the radiographs of the lateral thoracic spine were compared with age and sex matched controls obtained from accident service patients whose films had been recorded as normal. The radiographs of two controls and one patient taking etretinate were placed on a viewing screen. Five radiologists, ranging from a 1st year registrar to a consultant specializing in skeletal disorders, were asked to select the film most likely to show changes of hyperostosis. Only those cases correctly selected by all five were classified as abnormal.

A contingency table was constructed comparing each of the diagnostic groups (psoriasis and DOK), separating unaffected patients from those who showed evidence of hyperostosis either on radiographs or scintigraphy. The Chi-squared statistic was calculated from this table.

Within each diagnostic group further tables were constructed to analyse the relationship between hyperostosis and the total dose, the mean dose/kg/day and the duration of treatment. As these tables involved small numbers, Fisher's exact test was applied.

RESULTS

Thirteen patients with a congenital or inherited disorder of keratinization and 10 patients with psoriasis were studied. The age range was 18 to 60 years (mean 41·6). Duration of treatment varied from 21 to 75 months (Table 1).

One patient was thought to have a borderline scintigraphic examination (Patient 19). Review by both radiologists reclassified this case as abnormal.

Of the 13 DOK patients, four showed areas of activity on bone scintigraphy not explicable by degenerative disease (Figs 1 and 2). In three patients, areas outside the spine were found to be affected by hyperostosis on plain radiographs (Fig. 3). Based on independent selection by all five radiologists, four patients with a congenital or inherited disorder of keratinization showed hyperostosis on radiographs of the thoracic spine (Figs 4 and 5). Seven patients gave a history of musculoskeletal pain.

Out of 10 patients with psoriasis, five showed abnormal scintigraphy not explicable by degenerative disease. In three patients, plain radiographs showed hyperostosis in areas outside the thoracic spine. In five cases, all five radiologists considered that the lateral thoracic spine radiograph showed hyperostosis. Seven patients gave a history of musculoskeletal pain.

The commonest site of hyperostosis outside the spine was the knee (five out of eight sites) (Figs 6, 7 and 8).

In total, 14 patients described musculoskeletal symptoms, 11 of these had signs of hyperostosis. Only one patient had sought medical advice for his symptoms (Patient 17).

Two patients with psoriasis had significantly elevated 24-h urinary calcium levels. Six patients, three with DOK and three with psoriasis, showed low levels. Serum chemistry, liver function tests and haematological studies were all normal.

There was no significant difference between the numbers of affected and unaffected patients in each diagnostic group ($P > 0·50$). In those patients with a congenital or inherited disorder of keratinization there was no significant difference between the affected and unaffected patients with respect to the total dose ($P > 0·1$), the mean dose/kg/day ($P > 0·1$) or the duration of treatment ($P > 0·1$). Similarly for those with psoriasis there was no significant difference

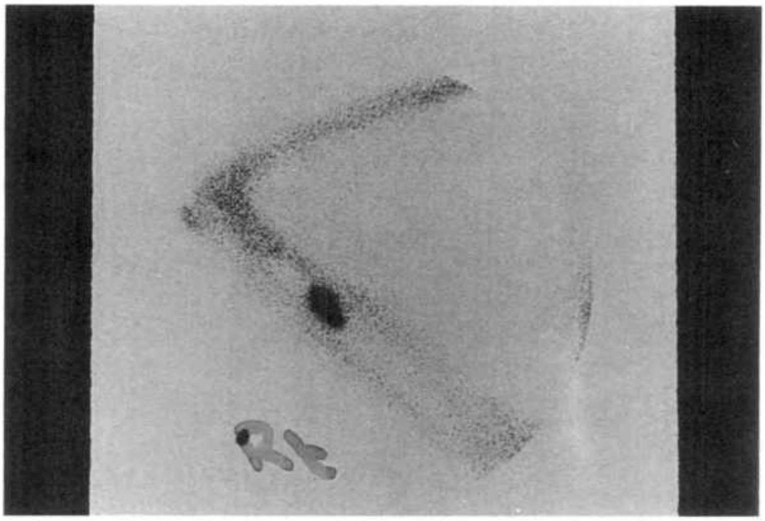

FIGURE 1. An area of marked increase in activity on scintigraphy in the mid forearm. (Patient 17).

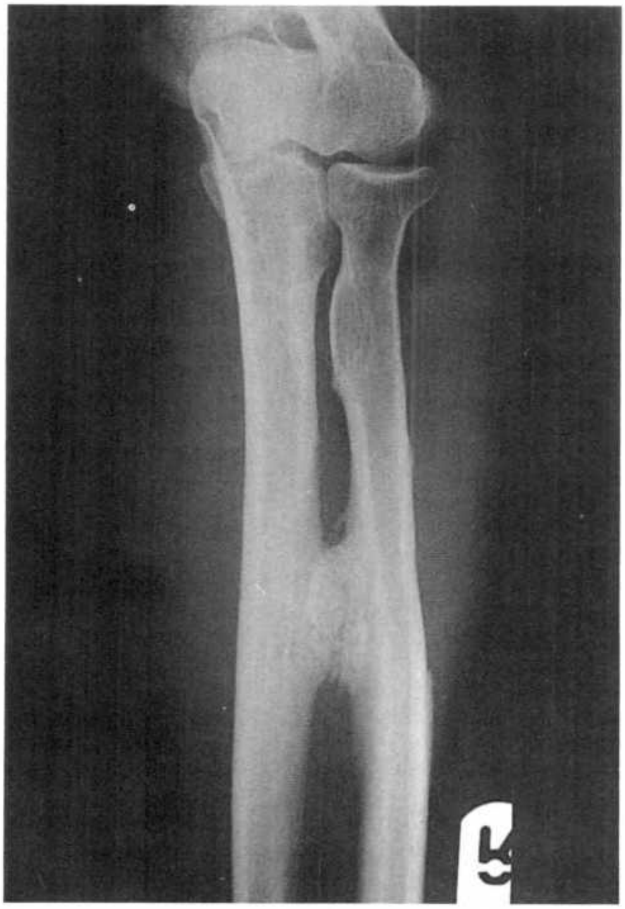

FIGURE 2. Plain radiograph of the patient in Figure 1 showing a florid area of hyperostosis in the interosseous membrane. (Patient 17).

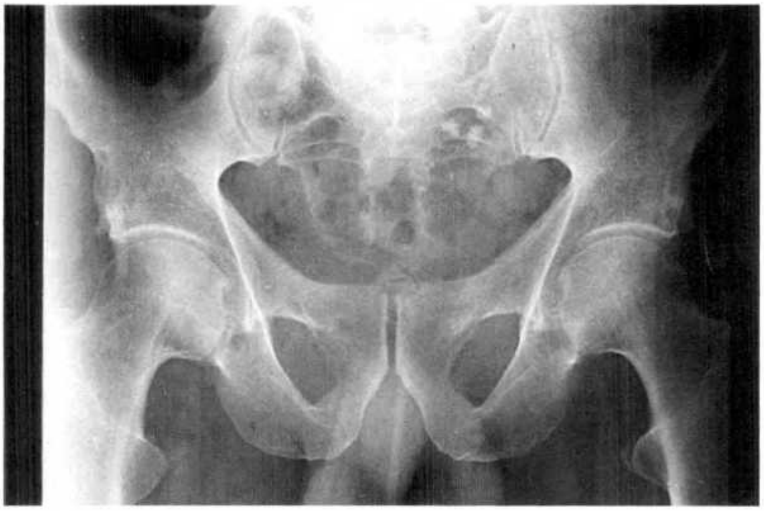

FIGURE 3. Areas of new bone in the iliopsoas insertions at both lesser trochanters and at the anterior inferior iliac spines. (Patient 17).

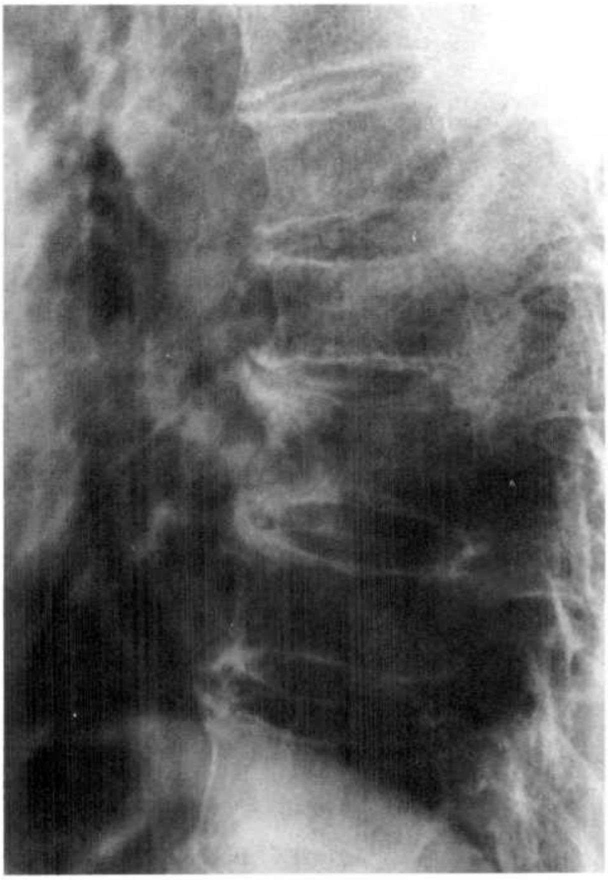

FIGURE 4. A lateral radiograph of the thoracic spine showing early hyperostosis that might be considered normal in an older patient. (Patient 13).

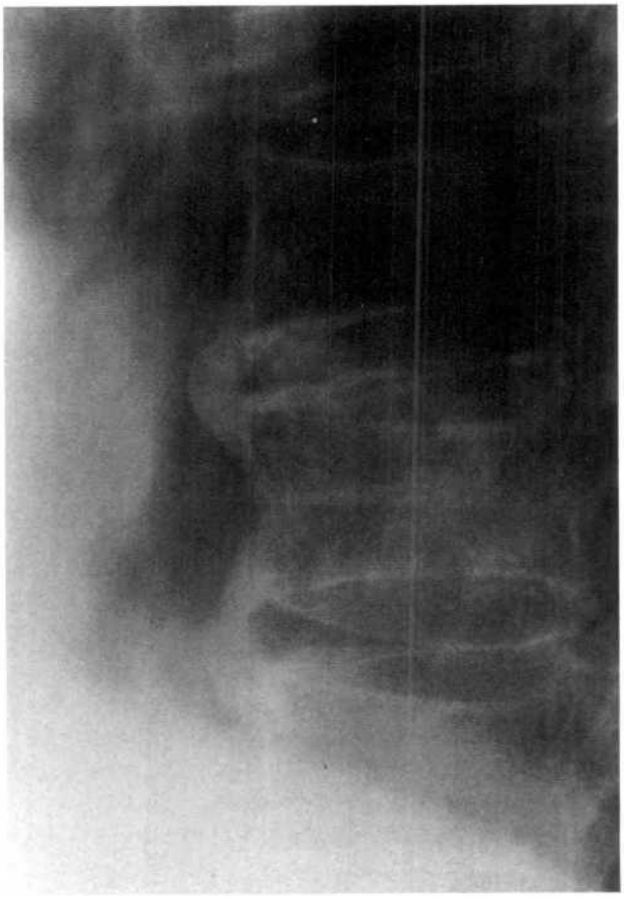

FIGURE 5. Florid flowing hyperostosis in the thoracic spine; abnormal at any age. (Patient 20).

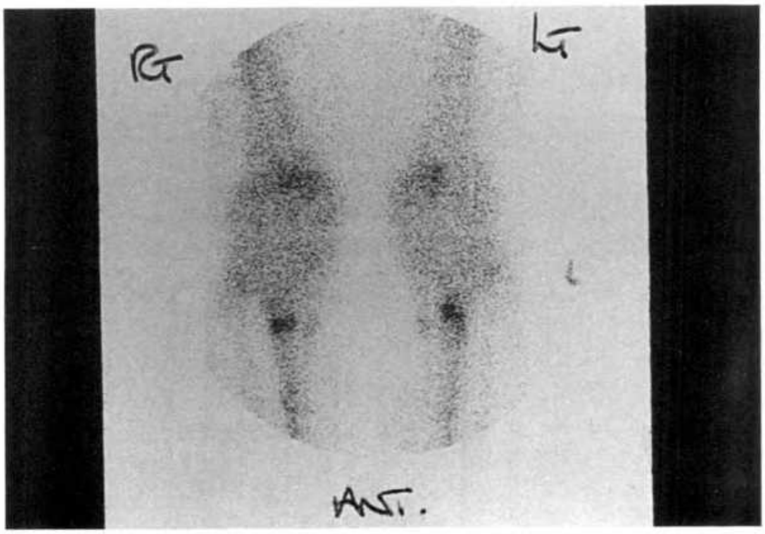

FIGURE 6. Excessive activity at both tibial tubercles on an anterior scintigram of both knees. (Patient 7).

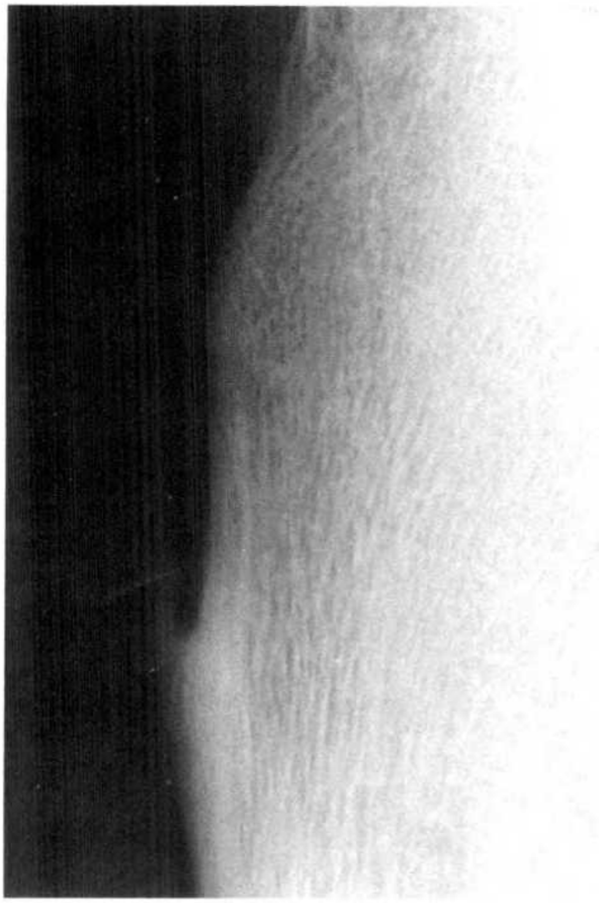

FIGURE 7. Prominent tendon insertions at the tibial tubercle in the patient in Figure 6. (Patient 7).

between those with and without skeletal effects with respect to total dose ($P > 0{\cdot}1$), mean dose/kg/day ($P > 0{\cdot}1$) and duration of treatment ($P > 0{\cdot}05$).

DISCUSSION

The results demonstrate that detectable hyperostosis is a fairly common finding in patients on long-term etretinate therapy. Musculoskeletal symptoms are common in the normal population and although the results suggest that there was a high incidence of symptoms related to administration of etretinate, with the exception of Patient 17, none of the study group had complained of these symptoms to their dermatologist or general practitioner. Even the patient who complained of stiffness in the forearms preferred this to what he considered would be the inevitable deterioration in his psoriasis if he were to stop taking etretinate. We do not know whether these patients will inexorably deteriorate, nor may we be certain that withdrawal of the drug would lead to a reversal of the hyperostosis.

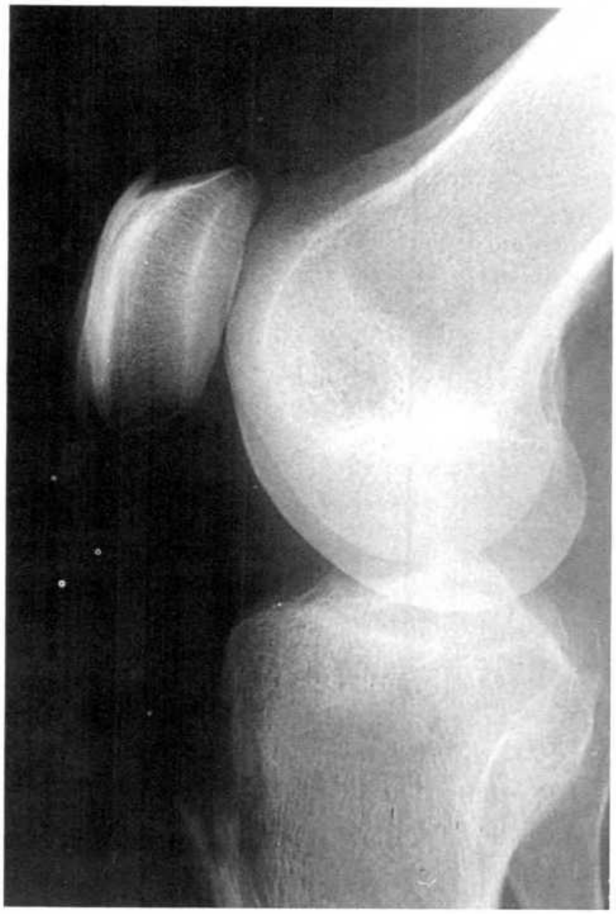

FIGURE 8. Hyperostosis at the tibial tubercle and patella entheses. (Patient 14).

We were unable to show any significant association between the bone side-effects and the quantity of drug or the duration of therapy. It may be that a larger sample would show some relationship, especially if patients on shorter periods of treatment were included. There is clearly a case for the serial examination of patients starting to take etretinate, continuing through a prolonged period of therapy.

Bone scintigraphy was disappointingly insensitive to the changes of hyperostosis and we cannot support the suggestion that it is a better screening test than plain radiography.[18] Patients with a significant degree of bone change would have been missed if this had been used as the only technique. The lateral thoracic spine radiograph is also less than ideal; however, it is considerably cheaper than bone scintigraphy and is more comfortable for the patient. The effective dose equivalent for bone scintigraphy is 2·06 mGy compared with 0·35 mGy for a lateral radiograph of the thoracic spine. The risk of bone scintigraphy when compared with the risk of smoking is equivalent to a total of 24 cigarettes.

A plain chest radiograph is a poor method for examination of the thoracic and cervical spine and we do not agree with Melnick *et al.*[5] who recommend its use to monitor skeletal changes.

From our observations we would recommend annual lateral thoracic spine radiographs with additional films of any area that becomes symptomatic.

Interpretation of radiographs is subject to inter-observer error, although our comparison of the diagnoses made by radiologists of varied experience suggests that the less expert observer is more likely to over-diagnose than to miss an abnormality.

In keeping with other reports, the haematology, serum chemistry and liver function tests were normal. However, the abnormal urinary calcium measurements were an unexpected finding and cannot be satisfactorily explained. Further studies are required.

Our initial hypothesis was that the bone changes in psoriasis may mask the features of hyperostosis. Congenital and acquired disorders of keratinization are not known to cause an arthropathy. The similarity of the results from the two groups suggests that psoriatic patients may be studied adequately for hyperostosis by the methods that we describe.

After moderately prolonged periods of etretinate therapy hyperostosis is a common finding. It is not certain whether these effects are related to dose and duration of treatment. Pending further studies we would currently recommend an annual lateral thoracic spine radiograph supplemented by films of any symptomatic area. We have insufficient evidence to make any recommendations regarding urinary calcium.

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Skeletal toxicity with isotretinoin therapy: a clinico-radiological evaluation

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SUMMARY

Skeletal toxicity is known to occur with high doses of isotretinoin (> 2 mg/kg/day). We have attempted to evaluate the clinical significance and document the extent of musculoskeletal toxicity associated with a relatively low dose of isotretinoin (0·5 mg/kg/day) used in the treatment of severe acne. Radiographs of 120 patients were examined. Twelve per cent showed minor changes (four patients had spinal hyperostoses and 10 had calcaneal hyperostoses). None of the musculoskeletal changes we observed was clinically significant. Comparison with matched control X-rays showed 8% of the controls to have similar non-significant changes. Follow-up of 11 of the patients with abnormal X-rays showed minor deterioration in one patient, no change in four and improvement in six. Thus, doses of 0·5 mg/kg/day isotretinoin in such patients did not produce any significant long-term musculoskeletal changes. With increasing use of this beneficial drug in acne, radiologists and dermatologists should be aware of its skeletal toxicity.

Since 1976, synthetic derivatives of vitamin A have been used with benefit in acne and certain hereditary scaling disorders. Isotretinoin (13-*cis*-retinoic acid) is a synthetic retinoid that produces dramatic improvement in cystic acne when given orally.[1] It was expected that these vitamin A analogues would produce toxic effects on the musculoskeletal system and previous reports have documented the spectrum of radiographic changes in the skeletal system.[2] We have reviewed the radiographs of 120 patients who received isotretinoin for severe acne. Our aim was to assess the clinical significance of any skeletal changes noted.

METHODS

One hundred and twenty patients (66 male, 54 female) with severe acne were treated with a 4-month course of oral isotretinoin (10 patients received 0·1 mg/kg/day, 80 patients 0·5 mg and 30 patients 1·0 mg). The average age was 25·8 years (range 18–52). Each patient was specifically questioned about any joint or bone pain and examined for any evidence of arthritis or local bony

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tenderness. No patient had any clinical evidence of psoriasis, rheumatoid arthritis or Reiter's disease. We considered it unnecessary to test blood for rheumatoid factor or HLA antigens. Each patient was radiographed within 1 year of treatment. Using photofluorography with a 14-inch image-intensifier, to keep the radiation dose to a minimum, the following views were

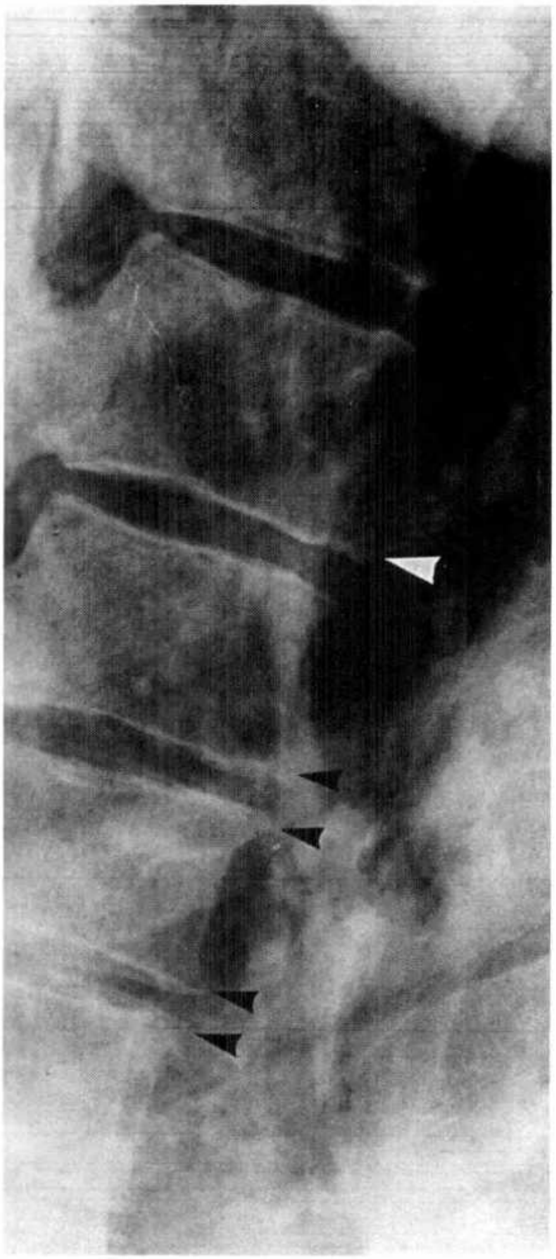

FIGURE 1. Lateral radiograph of thoracic spine. Hyperostoses (arrows) at insertion of anterior longitudinal ligament.

obtained: lateral views of both calcanea, antero-posterior views of both knees and lateral views of the dorsal and lumbar spine. Patients who had radiographic abnormalities were recalled for follow-up and similar radiographs obtained for comparison.

A control group of 120 patients, age- and sex-matched, was obtained from amongst patients radiographed in the Casualty Department for what proved to be an insignificant soft tissue injury. Comparable views of the spine, knees and calcanea were reviewed. These patients were not recalled for interview and so we do not know whether they had psoriasis, rheumatoid arthritis or Reiter's disease, but examination of their notes did not reveal any evidence of joint problems.

Each radiograph was reviewed blind by two independent radiologists and any disagreement in interpretation was resolved by joint viewing.

RESULTS

Fourteen patients in the study group (12%), were noted to have some radiographic abnormality. All of these patients were young (average age 26 years). Most of these abnormalities were subtle

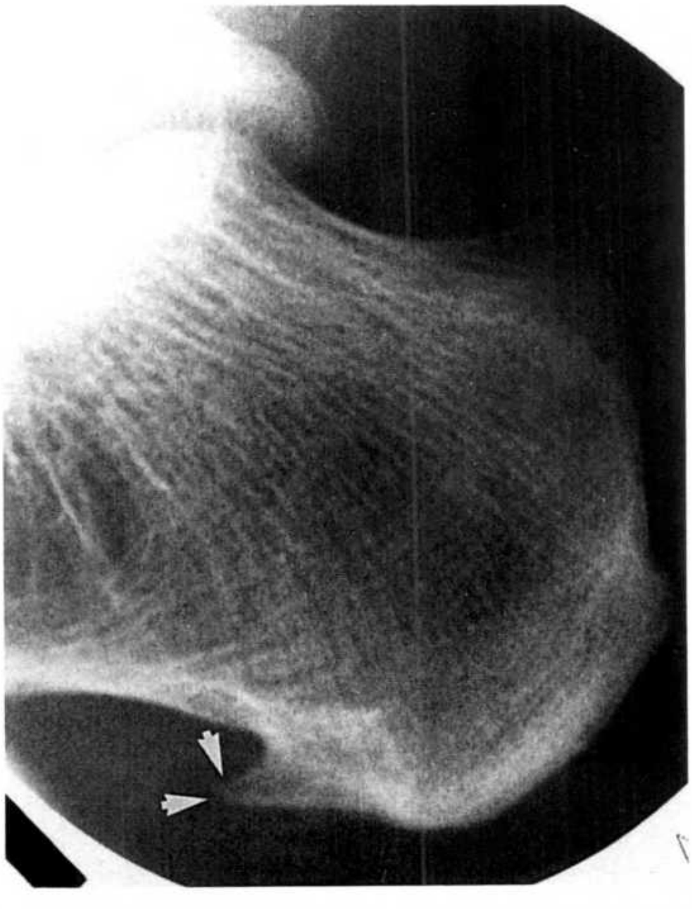

FIGURE 2. Lateral view of calcaneum. Hyperostosis (arrows) at insertion of plantar fascia.

TABLE 1. Numbers of patients and controls with abnormal radiographs

	Patients ($n = 120$)	Controls ($n = 120$)
Spinal hyperostoses	4	3
Calcaneal hyperostoses		
Plantar	6	3
Achilles tendon	4	2

and consisted of small hyperostoses at the insertions of the anterior spinal ligament (in 3%), the plantar fascia (in 5%) or the Achilles tendon (in 3%) (Figs 1, 2 and 3). In the knees, abnormalities of the tibial spines proved too insignificant and too subjective for worthwhile analysis. No patient had abnormalities at more than one site. Eight of the control group (7%) were noted to have hyperostoses at these sites (Table 1). Statistical analysis, using Chi-squared and Fisher's exact probability tests, showed no significant difference between the patient and control groups. The radiological changes were not dose-related.

Arthralgia was a frequent complaint of many patients (27%) whilst on treatment, but this decreased and usually disappeared in all after stopping treatment. The symptoms of mild discomfort with exertion were sporadic and in no way limited the patient's activities.

Five of the patients who did have skeletal changes complained of mild arthralgia whilst on therapy, but only one of these had pain at the site of abnormality (dorsal spine).

The 14 patients found to have evidence of hyperostoses were recalled for follow-up 18 months

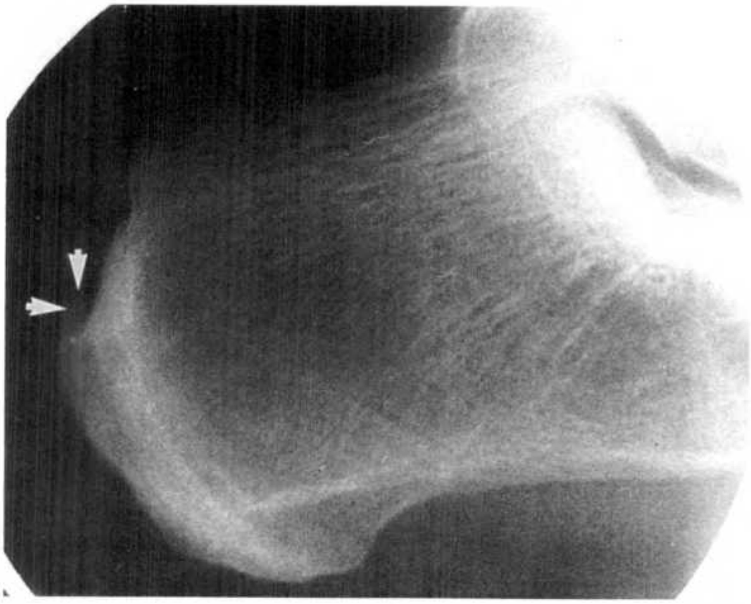

FIGURE 3. Lateral view of calcaneum. Hyperostosis (arrows) at insertion of Achilles tendon.

TABLE 2. Results of follow-up on the patients originally noted to have abnormalities

	No. of patients			
	Number attending for follow-up	Improved	No change	Deteriorated
Abnormal spine ($n=4$)	4 (100%)	2	1	1
Abnormal calcanea ($n=10$)	7 (70%)	4	3	—

after the original X-rays had been taken. Eventually, further comparable radiographs were obtained on 11 of these. Only one patient showed radiographic deterioration (Table 2) and none was clinically symptomatic. In six patients there was a definite improvement and in four the X-ray appearances were unchanged.

DISCUSSION

Since the introduction of oral isotretinoin into clinical use for the treatment of acne, the clinical results have been very gratifying.[3] Short-term use produces sustained remission in severe refractory acne.[1] In humans, the toxic effects of vitamin A on the musculoskeletal system have been well chronicled[4–6] and these changes are now being seen in association with the systemic long-term use of synthetic vitamin A derivatives such as isotretinoin. Unlike the changes associated with natural vitamin A, the musculoskeletal response to these newer derivatives seems to occur mainly in the axial skeleton and in the entheses.

Early reports of isotretinoin toxicity related to prolonged use, in relatively high dosage, for the treatment of various keratinization disorders. It was not anticipated that the lower dosage and much shorter periods of treatment used in acne would cause similar skeletal changes, and hence the present study was retrospective.

We recognise that our radiographic survey did not include all possible sites of hyperostosis, but our reviews of the literature suggested that views of the spine, knees and calcanea would be most relevant. Furthermore, given that we were examining a group of young patients it was considered important to minimize radiation dosage, particularly as with increasing use of isotretinoin further radiographic monitoring of these and other patients is likely. We were able to identify hyperostoses in the spine and calcanea, but we found the changes said to occur at the knees (hyperostoses of the tibial spines) too subjective to be of value and hence we did not include them in our results.

Only 14 (12%) of our patients had any evidence of hyperostoses and none of these were clinically significant. This accords well with the results of Kilcoyne *et al.*[7] who noted spinal hyperostoses in 10% of their patients. These patients received a higher dose of isotretinoin (1·0 to 2·0 mg/kg/day) for their acne than did our patients. In our patients the X-ray changes were not dose related.

Arthralgia associated with normal radiographs is a recognised complication of isotretinoin therapy[8] and this was borne out in our study. It could be argued that the abnormalities around the calcaneum may be explained by certain forms of seronegative arthritis, but the spinal changes bear a resemblance to diffuse idiopathic skeletal hyperostosis (DISH). However, the latter condition typically occurs in the elderly, involves multiple sites and shows very much more exuberant hyperostosis than the small spurs noted in isotretinoin toxicity.

Prospective studies are in progress to evaluate the long-term toxicity of low dose isotretinoin therapy. Isotretinoin is now a commonly used drug for severe acne. Dermatologists may wish to monitor its toxicity and hence radiologists should be aware of the nature of its skeletal side-effects. So far, we have not encountered any long-term serious side-effects, and any possible skeletal changes related to isotretinoin therapy are minimal. Its clinical efficacy would seem to outweigh any of the observed skeletal side-effects. Nevertheless, it may be worthwhile to perform pre-treatment X-rays in risk groups. It is, thus, our policy to X-ray patients over 35 years, those who have pre-isotretinoin therapy symptoms or signs and those patients who require a second or subsequent course of the drug.

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A comparative study of benzoyl peroxide and clindamycin phosphate for treating acne vulgaris

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SUMMARY

A water based 5% benzoyl peroxide gel (Benzac® W5) was compared with topical 1% clindamycin phosphate solution (Cleocin·T®) in the treatment of acne vulgaris using a randomized, investigator blind study design. Lesion counts were significantly reduced in both treatment groups over the 12-week study period; however, the reduction of total lesions produced by benzoyl peroxide gel was significantly greater than that produced by clindamycin phosphate ($P < 0.05$). Clindamycin phosphate had a milder effect on the skin surface in terms of peeling and drying than the benzoyl peroxide gel.

A recent Medline literature search from 1980 to 1987 revealed 65 English language publications involving the study of topical antibiotics, 36 involving benzoyl peroxide, and 10 comparing topical antibiotics with benzoyl peroxide gels for the treatment of acne vulgaris. Until recently there have been no reported studies comparing benzoyl peroxide with 1% clindamycin phosphate in acne vulgaris. A study reported by Tucker *et al.*,[1] concluded that the effectiveness of treatment did not differ significantly between the benzoyl peroxide gel they studied and clindamycin phosphate. The effectiveness of benzoyl peroxide has been related to its inhibition of the *Propionibacterium acnes* population[2,3] and its ability to decrease comedones,[4] while topical clindamycin phosphate has been shown to reduce cutaneous *P. acnes* numbers only.[5]

The present study was undertaken to assess and compare the efficacy of benzoyl peroxide and the most popular topical antibiotic, clindamycin phosphate,[6] in the treatment of acne vulgaris and to compare the skin surface parameters of oiliness, erythema, peeling and dryness.

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METHODS

A total of 60 patients with mild to moderate acne vulgaris, 34 females and 26 males (mean age 19·8 years, range 16–25 years), entered the study. They were randomly assigned to one of two treatment groups, benzoyl peroxide or clindamycin phosphate, consisting of 30 patients each. The study was conducted during 4 consecutive months from September through to December in Salt Lake City, Utah. Patients entering the study had more than 20 facial lesions, but no nodular-cystic lesions, had not received systemic or topical acne treatment in the previous 14 days, had not been under the care of a dermatologist for acne during the previous month and had no underlying diseases or other dermatological conditions. Following the initial evaluation, patients returned for four follow-up visits at 2, 4, 8, and 12 weeks. Informed consent was obtained from each patient or his or her guardian or both.

The investigator was blind as to which treatment group the patients belonged to and the patients were instructed not to discuss with the investigator, any aspect of the treatment to which they were assigned. At each visit total facial lesion counts, excluding the nose, were recorded. All lesion counts were made by the same investigator. Individual totals for non-inflamed lesions (open and closed comedones) and inflamed lesions (papules and pustules) were obtained. In addition, the patients' facial skin was rated clinically for degree of oiliness, erythema, peeling and dryness. Oiliness was evaluated by both visual appearance and feel and scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). Erythema was scored as 0 (none), 1 (barely perceptible), 2 (definite), or 3 (intense). Peeling was defined as the shedding of scales and scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). Dryness was defined as a brittle or rough sensation on palpating the skin and scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). All observations were recorded on study sheets and kept separate from the medical chart which contained the medication and compliance records.

TABLE 1. Changes in numbers of non-inflamed lesions during the study in 30 patients treated with benzoyl peroxide and 30 treated with clindamycin phosphate

	Treatment period				
	Baseline	Week 2	Week 4	Week 8	Week 12
Benzoyl peroxide					
No. of lesions: Mean ± SD	74·6 ± 77·1	58·2 ± 60·2	50·4 ± 60·3	37·3 ± 42·7	28·3 ± 32·3
Range	6–274	6–248	2–250	1–174	0–147
Percentage change from baseline		−22·0%	−32·4%	−50·0%	−62·9%
P		0·009	<0·001	<0·001	<0·001
Clindamycin phosphate					
No. of lesions: Mean ± SD	56·0 ± 50·9	53·9 ± 50·9	41·0 ± 44·1	36·5 ± 43·8	35·2 ± 46·0
Range	7–253	2–246	1–211	0–219	0–222
Percentage change from baseline		−3·8%	−28·6%	−34·6%	−37·1%
P		NS	0·002	<0·001	<0·001
Difference between treatments					
P	NS	<0·05	NS	<0·05	<0·001

NS = Not significant

In addition to the study medications, each patient was provided with a nonmedicated lipid-free cleanser (Cetaphil® lotion) for cleansing the face. The cleanser was used twice daily prior to applying the study medication using the fingers to massage it gently into the skin and then removing the excess by patting it off with a soft cotton towel. This cleanser was chosen to minimize dryness during the autumn and winter season[7] and to eliminate variability in cleansing that might affect the results of the study. Patients in each treatment group applied the respective medication to the face twice daily (morning and evening).

Statistical analysis

A *t*-test was used to compare pre-study lesion counts in the two groups. Repeated measure analyses of variance were performed on changes from baseline for comedones, inflammatory lesions (papules and pustules) and total lesions. Wilcoxon's rank sum test was used for analysis of skin condition parameters, oiliness, erythema, peeling and dryness.

RESULTS

All 60 patients completed the 12-week study, none dropped out or missed any visits. The two study groups were similar in age, and initial counts of non-inflammatory lesions, inflammatory lesions and total lesions. While the benzoyl peroxide group had larger numbers of non-inflammatory lesions at baseline, this difference was not statistically significant (Table 1). Sex ratio was the only statistically significant difference between the treatment groups with a disproportionately greater proportion of males (20:10) assigned to clindamycin phosphate and females (24:6) to benzoyl peroxide ($P < 0{\cdot}01$). Patient compliance with each of the treatment programmes was considered acceptable.

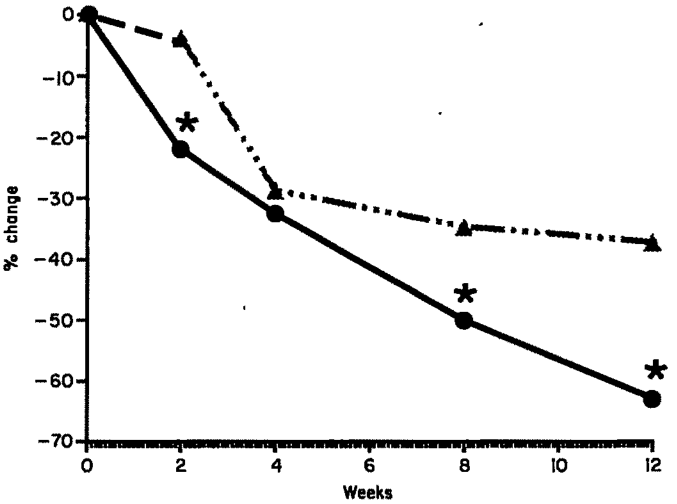

FIGURE 1. Mean percentage change from baseline in numbers of non-inflamed lesions in acne patients treated with benzoyl peroxide (●) or clindamycin phosphate (▲). * Significant difference between the two treatments.

TABLE 2. Changes in numbers of inflammed lesions during the study

	Treatment period				
	Baseline	Week 2	Week 4	Week 8	Week 12
Benzoyl peroxide					
No. of lesions: Mean ± SD	22·8±22·5	19·3±16·8	16·5±16·5	14·7±15·0	10·8±9·4
Range	2–102	3–77	2–65	0–71	1–44
Percentage change from baseline		−15·4%	−27·6%	−35·1%	−52·2%
P		NS	0·012	0·031	<0·006
Clindamycin phosphate					
No. of lesions: Mean ± SD	24·4±16·6	21·7±14·1	18·1±12·7	18·9±18·3	17·6±18·4
Range	4–63	3–56	3–57	2–86	1–78
Percentage change from baseline		−11·1%	−25·8%	−22·5%	−27·9%
P		NS	0·014	NS	<0·021
Difference between treatments					
P		NS	NS	NS	NS

NS = Not significant

TABLE 3. Changes in total numbers of lesions during the study

	Treatment period				
	Baseline	Week 2	Week 4	Week 8	Week 12
Benzoyl peroxide					
No. of lesions: Mean ± SD	97·4±93·6	77·5±73·8	66·9±73·2	52·1±56·0	39·1±38·1
Range	20–361	10–325	4–290	3–213	3–166
Percentage change from baseline		−20·3%	−31·3%	−46·5%	−59·8%
P		0·006	<0·001	<0·001	<0·001
Clindamycin phosphate					
No. of lesions: Mean ± SD	80·4±62·9	75·5±61·2	59·1±53·5	55·4±54·8	52·7±59·0
Range	21–309	11–290	8–247	2–239	1–258
Percentage change from baseline		−6·0%	−26·5%	−31·1%	−34·3%
P		NS	0·001	<0·001	<0·001
Difference between treatments					
P	NS	NS	NS	NS	0·005

NS = Not significant

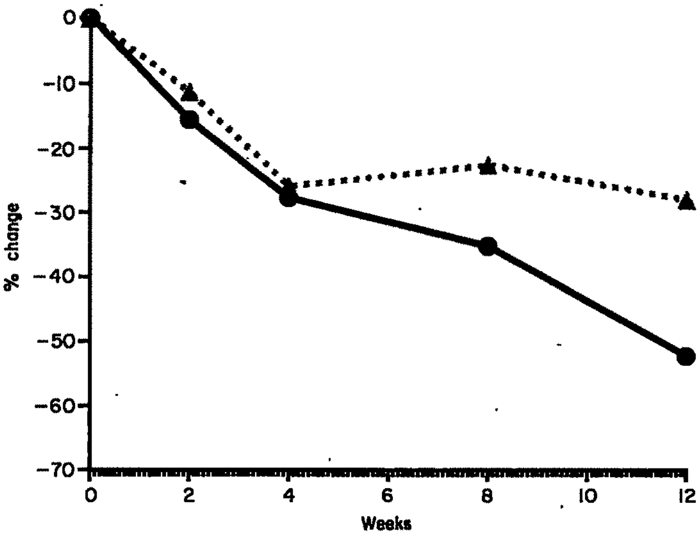

FIGURE 2. Mean percentage change from baseline in numbers of inflamed lesions in acne patients treated with benzoyl peroxide (●) or clindamycin phosphate (▲).

Lesion comparisons

Both treatments reduced comedones (non-inflamed lesions) throughout the study (Table 1), but the difference between the benzoyl peroxide and clindamycin groups was significant at 2 weeks ($P < 0.05$), 8 weeks ($P < 0.05$), and 12 weeks ($P < 0.05$) in favor of benzoyl peroxide (Fig. 1).

Both treatments were effective in reducing inflamed lesions, and the results were not

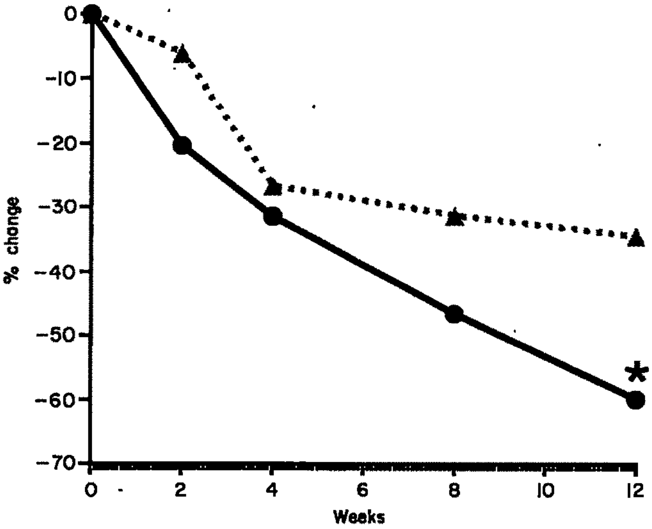

FIGURE 3. Mean percentage change from baseline in total numbers of lesions in acne patients treated with benzoyl peroxide (●) or clindamycin phosphate (▲). * Significant difference between the two treatments.

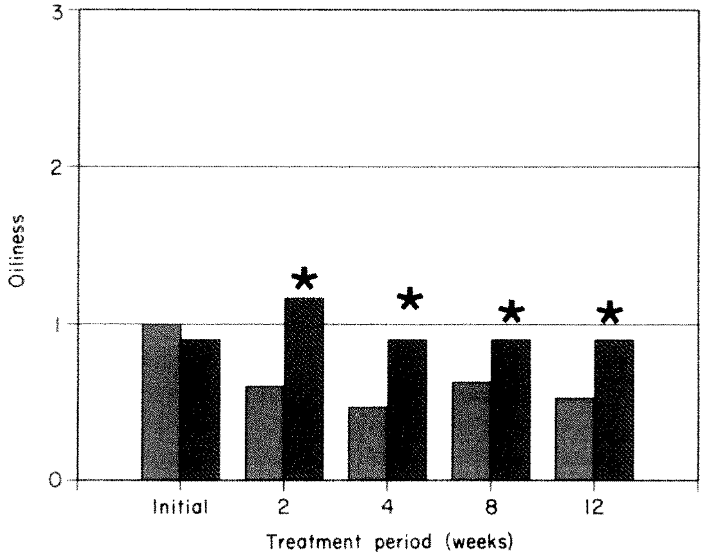

FIGURE 4. Mean clinical scores for oiliness in acne patients treated with benzoyl peroxide (▨) or clindamycin phosphate (■). * Significant difference between treatments, $P<0\cdot05$.

significantly different in the two groups (Table 2). Benzoyl peroxide tended to be more effective, with a 52% reduction in inflammatory lesions at 12 weeks compared 2 with a 28% reduction with clindamycin phosphate (Fig. 2).

Total lesions in both groups were reduced from Week 4 onwards (Table 3), but significant differences were not seen between the two groups until Week 12 ($P<0\cdot05$) with benzoyl

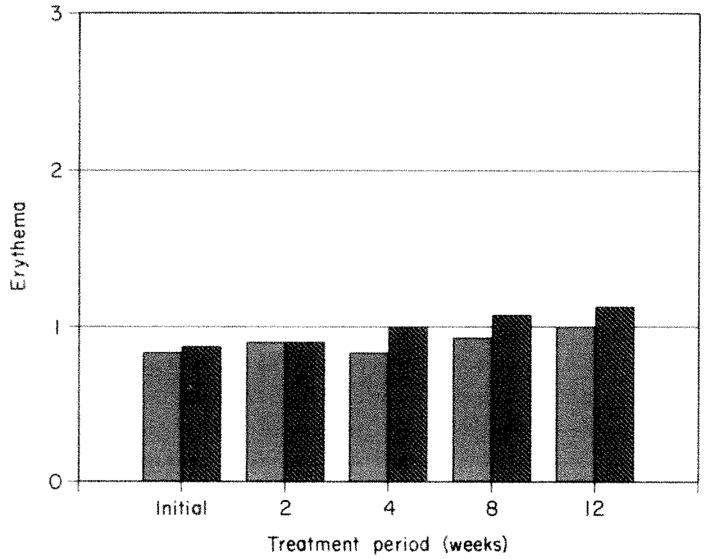

FIGURE 5. Mean clinical scores for erythema in acne patients treated with benzoyl peroxide (▨) or clindamycin phosphate (■).

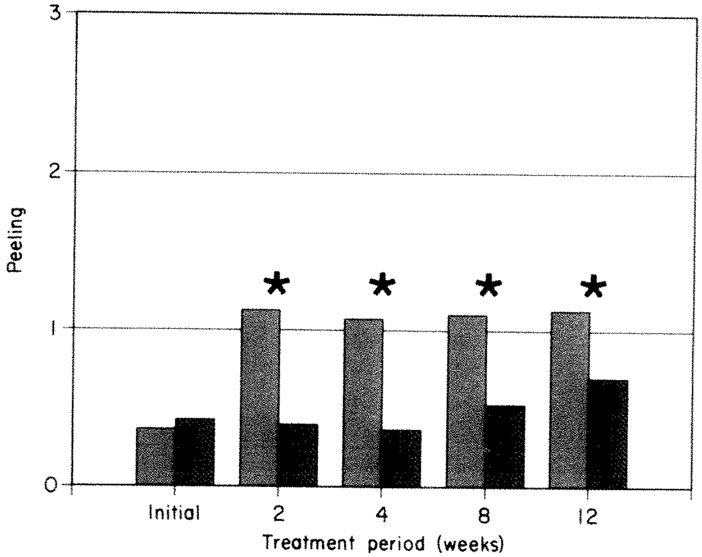

FIGURE 6. Mean clinical scores for peeling in acne patients treated with benzoyl peroxide (▨) or clindamycin phosphate (▩). * Significant difference between treatments, $P < 0.05$.

peroxide giving a 59·8% reduction in total lesions compared with 34·3% with clindamycin phosphate (Fig. 3).

Skin surface evaluation

No adverse effects were reported with either treatment. Oiliness remained relatively constant with clindamycin phosphate but decreased significantly with benzoyl peroxide ($P < 0{\cdot}05$)

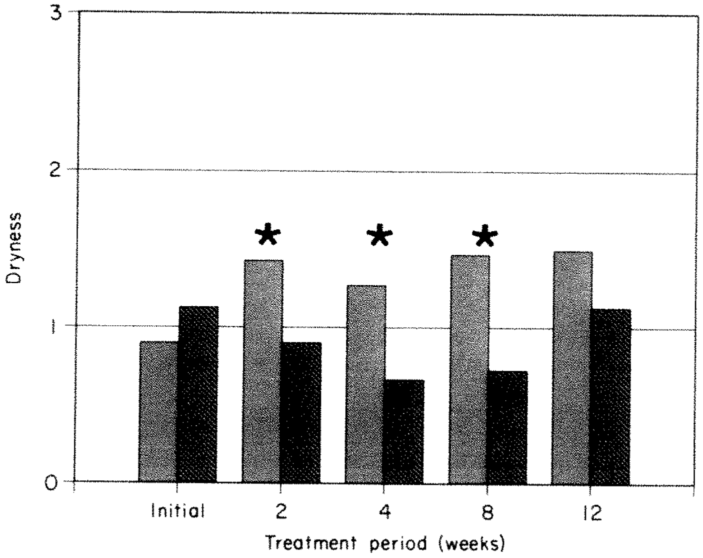

FIGURE 7. Mean clinical scores for dryness in acne patients treated with benzoyl peroxide (▨) or clindamycin phosphate (▩). * Significant difference between treatments, $P < 0{\cdot}05$.

(Fig. 4). No difference in erythema was detected between the two groups throughout the study (Fig. 5). Peeling was significantly less in the clindamycin group ($P < 0{\cdot}05$) (Fig. 6). Dryness was also significantly less in the clindamycin group at 2, 4 and 8 weeks ($P < 0{\cdot}05$) (Fig. 7), but there was no significant difference between the two groups at week 12.

DISCUSSION

Benzoyl peroxide gels and clindamycin phosphate are popular treatments for the topical management of acne vulgaris. They both have an antimicrobial affect and the benzoyl peroxide gels have also been shown to have an appreciable comedolytic effect on acneform lesions in rabbits, an effect which topical antibiotics were not known to possess.[5] Based on our findings, benzoyl peroxide might be expected to have a more rapid and profound effect in reducing acne lesions than clindamycin phosphate because it appears to possess two mechanisms of action rather than one.

The results of this study demonstrated a rapid reduction of lesions, especially comedones, by Week 2 of treatment with benzoyl peroxide. This reduction of comedones supports the possibility that benzoyl peroxide may reduce clinically evident comedones in humans. Earlier studies did not show comedolytic activity against microcomedones in a human model[8] or on the faces of acne patients,[9] however, a previous study utilizing an animal model did show a reduction in comedones with benzoyl peroxide applications.[4] The clindamycin group did not achieve a comparable reduction in comedonal lesions until the 4th week. While both treatments were effective in significantly reducing the total lesion counts, the benzoyl peroxide gel had produced a significantly larger effect by Week 12 than had clindamycin phosphate. Thus, 5% benzoyl peroxide gel proved superior to 1% clindamycin phosphate as a topical therapy for acne vulgaris.

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Double-blind comparison of a dithranol and steroid mixture with a conventional dithranol regimen for chronic psoriasis

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SUMMARY

Twenty four of 30 patients with chronic plaque psoriasis completed a double-blind randomized paired comparison of dithranol in Lassar's paste versus dithranol and fluocinonide in Lassar's paste. There was a more rapid reduction in severity of the psoriasis on the side treated with the dithranol and fluocinonide mixture, but no difference in the final time to clearance between the two sides. There were fewer burning episodes on the side treated with dithranol and fluocinonide and greater staining of the skin. Of the 20 patients who were subsequently followed up for up to 3 months, 11 had relapsed more on the side treated with dithranol and fluocinonide, two on the side treated with dithranol alone and in seven there was no appreciable difference. It was concluded that the addition of a topical steroid to a conventional dithranol regimen produced more rapid initial clearance and less burning, but more rapid relapse of the psoriasis.

Previous studies of a dithranol and steroid regimen[1] and a tar and steroid regimen[2] for psoriasis both showed an earlier relapse when steroid was included. However, the study by Seville[1] had serious methodological flaws because the control group was taken from retrospective data and was not matched for extent of psoriasis, nor were the treatments randomized or given blind. Dithranol 0·25% with salicylic acid 1% in emulsifying ointment B.P. was alternated daily with 0·1% betamethasone valerate ointment under polythene occlusion for 18 h and this regimen is rarely used now in clinical practice. In the present study, we chose to compare the effects on clearing and rate of relapse, of dithranol in Lassar's paste with dithranol and fluocinonide in Lassar's paste because these mixtures had been in regular use in our department for the last 20 years and there was a clinical impression that the patients treated with the dithranol and fluocinonide mixture did better.

METHODS

Patients with chronic plaque psoriasis involving the arms, legs and trunk who had been admitted to the General Hospital, Birmingham, for routine dithranol treatment were randomized to

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receive dithranol in Lassar's paste on one side of the body and dithranol and fluocinonide on the other. The face, neck and flexures were treated with 0·05% clobetasone butyrate cream (Eumovate®, Glaxo) and the scalp with ung. cocois co. (St John's Formula, Martindale). One part Metosyn® ointment (Stuart Pharmaceuticals Ltd) was added to four parts dithranol in Lassar's paste (zinc oxide 24 g, salicylic acid 2 g, starch 24 g, white soft paraffin 50 g) to achieve working concentrations of 0·01% fluocinonide and 0·1, 0·25 and 0·5% dithranol. One part white soft paraffin was added to four parts dithranol in Lassar's paste to achieve the same consistency in the treatment which did not include steroid. All patients started treatment with 0·1% dithranol twice daily and any subsequent changes in strength were made equally on both sides, as determined by the clinical response. Burnt areas were treated with a mixture of white soft paraffin and liquid paraffin in equal parts until dithranol could be restarted. Randomization was done by the Pharmacy and neither clinicians, nursing staff nor patients were informed of the allocations until the full study had been completed.

Surface area of involvement was assessed on admission by two clinicians using the 'rule of nines' and the severity of psoriasis was estimated by the method described by Downey and Finlay[3] where three pairs of symmetrical plaques on the arms, body and legs were graded from 0–3 for erythema, thickness and scaling, giving a maximum total score of 27 for each side. Severity assessments were subsequently repeated, after the morning bath and before treatment was applied, every Monday afternoon until the patient was discharged from the ward. At the time of discharge the patients were asked to complete 10 cm visual analogue scales to indicate their impression of overall improvement for each side of the body, and the degree of skin staining on each side was noted on a four point scale by the clinician. In addition, the nursing staff kept a full record during treatment of the times that dithranol had to be omitted because of burning.

Patients were instructed not to use dithranol at home and were followed up at monthly intervals for up to 3 months until there was an obvious difference in the extent of psoriasis on one side of the body, or the total area of relapse exceeded 5%. At each visit the total area of involvement was assessed by the same two clinicians using the 'rule of nines' and the percentage involvement of the left and right sides of the body, excluding the head, neck and genitalia, were also recorded.

RESULTS

Thirty patients were recruited (10 male, 20 female; mean age 40·8 years, range 16–79). Five were skin type I, 9 type II, 12 type III, 2 type IV and 2 type V. Mean surface area of involvement on admission was 18·4 ± 16·1% and mean duration of inpatient treatment was 17·7 days (range 7–28).

Twenty four patients continued paired treatments until ward discharge and were included in the data analysis. Of the other six, three were changed to dithranol and fluocinonide all over because they had shown such a marked improvement on that side that it was thought to be unreasonable for them to continue the paired comparison any longer, one was intolerant of both treatments, one had to be started on methotrexate because of intolerance and poor response to both treatments and one became hypomanic and was unable to complete the study.

The mean severity scores for the two treatments at the weekly assessments are shown in Figure 1. It can be seen that there was no difference between the baseline scores for the two sides but at the second assessment there was a greater reduction in the severity score on the side treated with dithranol and fluocinonide (mean 7·8; range 4–12) than the side treated with dithranol alone (mean 10·4; range 7–14) $P < 0·01$ Wilcoxon signed rank test). However, by the 3rd and 4th weeks the difference was no longer significant.

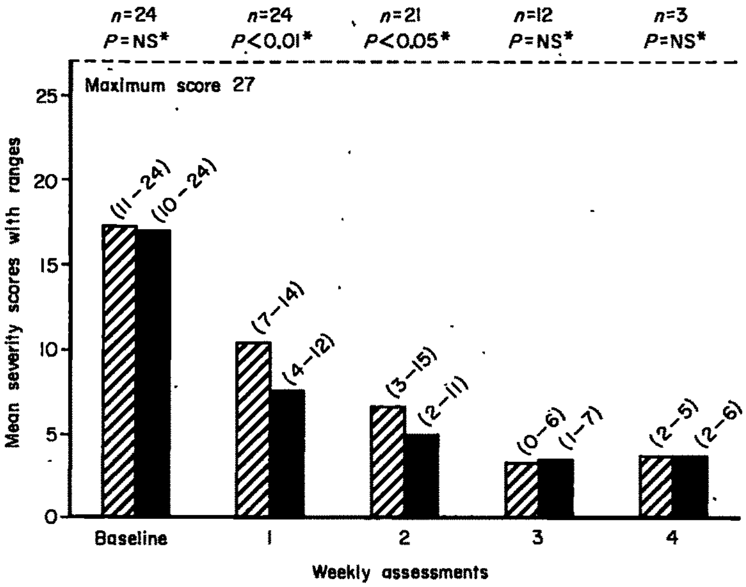

FIGURE 1. Change in mean severity scores during treatment with dithranol ▨ and dithranol plus fluocinonide ■. n = number of patients remaining in the study. * Wilcoxon signed rank test.

The patients' self-assessment using visual analogue scales showed no statistically significant preference between the dithranol treated side (mean 7·50; range 2–10) and the dithranol and fluocinonide treated side (mean 7·73; range 4–10) (Wilcoxon signed rank test).

The mean number of burning episodes with dithranol alone was 2·8 (range 0–9) and with dithranol and fluocinonide was 2·0 (range 0–9); ($P < 0·05$ Wilcoxon signed rank test).

Skin staining was more marked on the side treated with dithranol and fluocinonide than on the side treated with dithranol alone in 12 patients (Fig. 2), less marked in 1 and the same in 9 ($P = 0·002$; Sign test).

Twenty of the patients who completed the paired treatments were followed up for up to 3 months. At 1 month, eight patients were judged to have relapsed and were withdrawn from the study, at 2 months five had relapsed and at the final 3-month assessment only four were still clear. The degree of relapse was calculated by taking the percentage difference in area involved between the two sides divided by the total area involved, including scalp and genitalia. At the final follow-up assessment, 11 patients had at least 25% more involvement on the dithranol and fluocinonide treated side, two patients showed at least 25% more involvement on the side treated with dithranol alone and in seven there was no difference ($P = 0·011$, Sign test). The difference in relapse between the two sides is well illustrated by the patient shown in Figure 3.

Of the three patients who were withdrawn from the study because of their excellent initial response to the dithranol and fluocinonide mixture, one had relapsed sufficiently to need dithranol again after 1 month, one had remained substantially clear at 1 month and was discharged from the clinic and the other did not reattend for follow-up.

DISCUSSION

This study confirms that the addition of a topical steroid to a dithranol regimen results in significantly earlier relapse. However, it should be noted that patients treated with dithranol and fluocinonide had fewer burning episodes and a more rapid early clinical response. We have

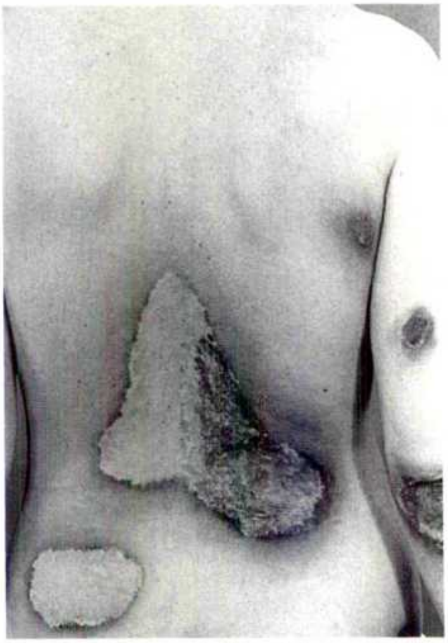

FIGURE 2. Increased skin staining on the side treated with dithranol and fluocinonide.

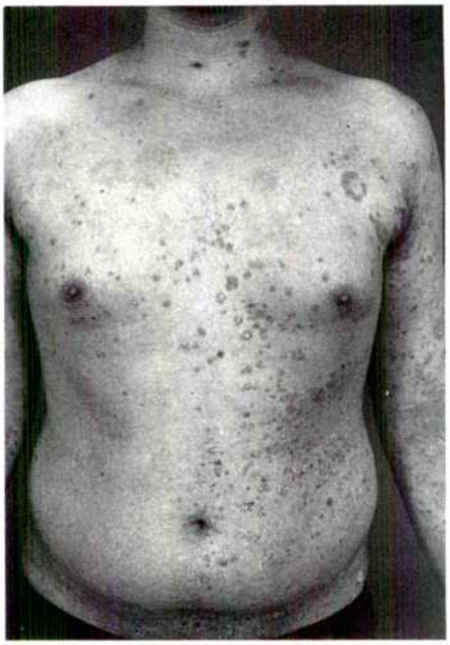

FIGURE 3. Earlier relapse on the side treated with dithranol and fluocinonide.

found that this mixture has a place in the management of patients who burn with low concentrations of dithranol, enabling them to tolerate treatment in the early stages before changing to dithranol in Lassar's paste with the aim of encouraging a longer remission.

Because of uncertainty about the stability of both dithranol and fluocinonide in the mixture, a shelf-life of 2 weeks was assumed in this study. No published data are available on the stability of dithranol and fluocinonide mixtures to our knowledge, although work is planned to clarify this point.

Skin staining is due to the formation of an oxidized dimer from dithranol. It is thought that this oxidation reaction occurs in epithelial mitochondria[4] although the drug will oxidize spontaneously when it comes into contact with any skin protein. Increased staining on the steroid treated side (which showed a more rapid clinical response) could, therefore, be due to enhanced degradation of the dithranol or reduced shedding of stratum corneum due to faster control of the hyperproliferative state.

ACKNOWLEDGMENTS

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Inhibition of 5α-reductase activity in human skin by zinc and azelaic acid

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SUMMARY

The effects of zinc sulphate and azelaic acid on 5α-reductase activity in human skin were studied using an *in vitro* assay with 1,2[^{3}H]-testosterone as substrate. When added at concentrations of 3 or 9 mmol/l, zinc was a potent inhibitor of 5α-reductase activity. At high concentrations, zinc could completely inhibit the enzyme activity. Azelaic acid was also a potent inhibitor of 5α-reductase; inhibition was detectable at concentrations as low as 0·2 mmol/l and was complete at 3 mmol/l. An additive effect of the two inhibitors was observed. Vitamin B6 potentiated the inhibitory effect of zinc, but not of azelaic acid, suggesting that two different mechanisms are involved. When the three substances were added together at very low concentrations which had been shown to be ineffective alone, 90% inhibition of 5α-reductase activity was obtained. If this inhibition is confirmed *in vivo*, zinc sulphate combined with azelaic acid could be an effective agent in the treatment of androgen related pathology of human skin.

Skin is a target tissue for androgens and, as a result, increased androgen activity is accompanied by a well-defined skin pathology including hyperseborrhoea, acne and hirsutism or alopecia. In skin, as in many other target tissues, the reduction of testosterone to dihydro-testosterone (DHT) by the enzyme 5α-reductase is the most important of the enzymatic processes involved in androgen activity; indeed DHT is a more potent androgen than is testosterone, due to its greater affinity for the androgen receptor. The active DHT is formed at the target cell site and the enzyme 5α-reductase, therefore, acts as an amplifier of the androgen signal.[1] DHT is generally considered responsible for the stimulation of the sebaceous gland[2] and increased local formation of DHT has been documented in acne[3] and in hirsutism.[4] Any product capable of limiting local DHT production could represent, therefore, a potential therapeutic agent. The trace element zinc has been used in dermatology from the times of the Ancient Egyptians; only more recently has it been shown to reduce sebum secretion.[5] In addition it is involved in numerous enzyme systems[6] and has been reported to play a regulatory role in testosterone

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metabolism in the human prostate gland.[7] We have previously shown that zinc can inhibit, *in vitro*, the 5α-reductase of human skin.[8] Also, topical application of azelaic acid has been reported to have beneficial effects on acne vulgaris.[9] In the present study we have examined the *in vitro* effects of zinc and azelaic acid on testosterone metabolism and in particular, on 5α-reductase activity.

METHODS

Materials

1,2[^{3}H]-Testosterone (specific activity 60 mCi/mmol), [^{14}C]-testosterone (specific activity 60 mCi/mmol), [^{14}C]-dihydrotestosterone (specific activity 58 mCi/mmol) and [^{14}C]-Δ4-androstenedione (specific activity 57 mCi/mmol) were obtained from Amersham, France and purified on celite columns before use. [^{14}C]-androstanediols (3α and 3β Adiols) were prepared by sodium borohydrate reduction of [^{14}C]-DHT as previously described.[10] Unlabelled steroids were purchased from Sigma (St. Louis, MO, U.S.A) and crystallized in methanol before use. Stock solutions (10 mmol/l) were prepared in ethanol and stored at 4°C. NADPH was obtained from Boehringer Mannheim, France S.A. (Meylan) and diluted in buffer before use. All solvents and reagents were of analytical grade. Zinc sulphate, azelaic acid and pyridoxine (vitamin B6) were kindly provided by the Laboratoires Bailleul.

Tissue preparation

Foreskins from normal children (2–3 months old) undergoing circumcision were obtained, with informed consent from the parents, snap frozen in liquid nitrogen and stored at −80°C. They were then homogenized with a Polytron homogenizer (five 10s strokes) in ice-cold Krebs-Ringer phosphate buffer (120 mmol/l NaCl, 4·8 mmol/l KCl, 2·6 mmol/l $CaCl_2$, 1·2 mmol/l $MgSO_4$), pH 6·4 and used as a source of 5α-reductase.

Enzymatic assays

The assay for 5α-reductase activity in human skin homogenates has been reported previously.[11] Increasing concentrations of [^{3}H]-testosterone (9, 10, 15, 20, 30, 50, 100 and 500 nmol/l) were evaporated in glass tubes and NADPH (2·4 mmol/l) diluted in buffer was added; the reaction was initiated by the addition of an amount of homogenate corresponding to 10 mg of tissue and the volume adjusted to 1 ml with buffer. Incubations were carried out in a Dubnoff metabolic incubator at 37°C for periods of 5 to 30 min and stopped by the rapid addition of 10 ml of ethyl acetate/cyclohexane 1:1 (v/v). After addition of [^{14}C]-steroids to monitor recovery and unlabelled tracers for easy visualization, the steroids were extracted and evaporated to dryness. Tubes without homogenates, but containing the same amount of substrate, buffer and co-factors were always incubated in parallel to determine blank values. The metabolites were separated by thin-layer chromatography on silica gel, in chloroform/methanol 97·5:2·5 (v/v). Testosterone and Δ4-androstenedione were visualized under UV light (240 nm) and 5α-reduced steroids using iodine vapour. Steroids were scraped from the plate and eluted in ethanol and acetone. 3α- and 3β-adiols, not separated in this system, were eluted together. Samples were counted in a Packard 300 C liquid scintillation spectrometer with an efficiency of 68% for [^{14}C] and 30% for [^{3}H]. After correction for recovery and deduction of blank values, 5α-reductase activity was expressed as fmol (DHT + Adiols)/h/mg of tissue.

Effect of zinc and azelaic acid on 5α-reductase activity

Zinc sulphate was added to the incubation tubes at final concentrations of 0·5, 1·5, 3, 9 or

15 mmol/l diluted in 100 μl buffer, and the assay was performed as described above. Azelaic acid was added at final concentrations of 0·1, 0·2, 0·5 or 3 mmol/l diluted in 10 μl ethanol. The same amount of ethanol was also added to the control tubes. In some experiments, pyridoxin (vitamin B6) at a final concentration of 0·025% was also added to the incubation medium. These compounds were added separately or together in various concentrations in order to determine the minimal concentrations which gave a maximal inhibitory effect.

RESULTS

Preliminary experiments have shown that incubation for 5 min gave good estimates of the initial velocity of the reaction for substrate concentrations as low as 10 nmol/l (data not shown). We have used 20 nmol/l testosterone and 5 min incubations to study the effects of zinc and azelaic acid on 5α-reductase activity.

Effect of zinc and azelaic acid on 5α-reductase activity

The effects of increasing concentrations of zinc sulphate (1·5 to 15 mmol/l) and azelaic acid (0·1 to 3 mmol/l) on 5α-reductase activity were studied separately and the results are shown in Figure 1. In both cases, there was a dose-dependent inhibition of the enzyme activity; 98% inhibition was observed with 15 mmol/l $ZnSO_4$ and with 3 mmol/l azelaic acid. When the two compounds were added together to the incubation medium at concentrations expected to give 50–60% inhibition (3 mmol/l and 0·5 mmol/l, respectively), 95% inhibition was observed (data not shown).

Effect of vitamin B6 on zinc and azelaic acid inhibition of 5α-reductase activity

The addition of vitamin B6 (0·025%) to zinc sulphate (1·5 or 3 mmol/l) resulted in a two-fold increase in the inhibition of the enzyme activity (Fig. 2a). In contrast, vitamin B6 had no effect on 5α-reductase activity when added alone or together with azelaic acid (Fig. 2b). These results suggest that zinc and azelaic acid might inhibit 5α-reductase activity through two different mechanisms.

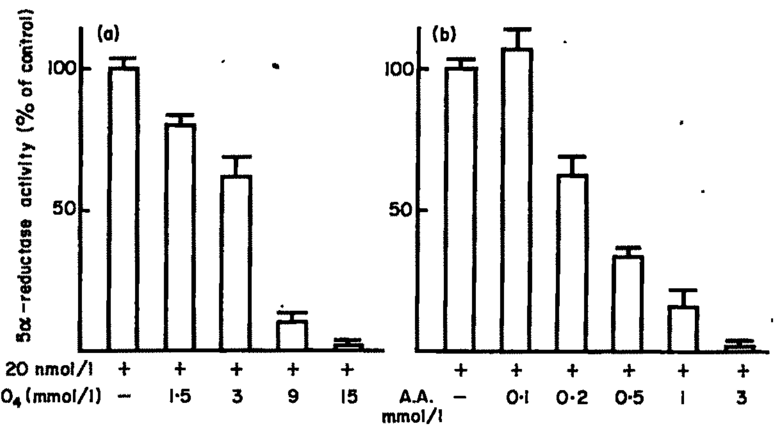

FIGURE 1. Effect of (a) $ZnSO_4$ and (b) azelaic acid (AA) on 5α-reductase activity in human skin homogenates. Results are expressed as percentages of controls without inhibitor. Values are means ± SD ($n = 6$). ^{3}H-T = ^{3}H-testosterone.

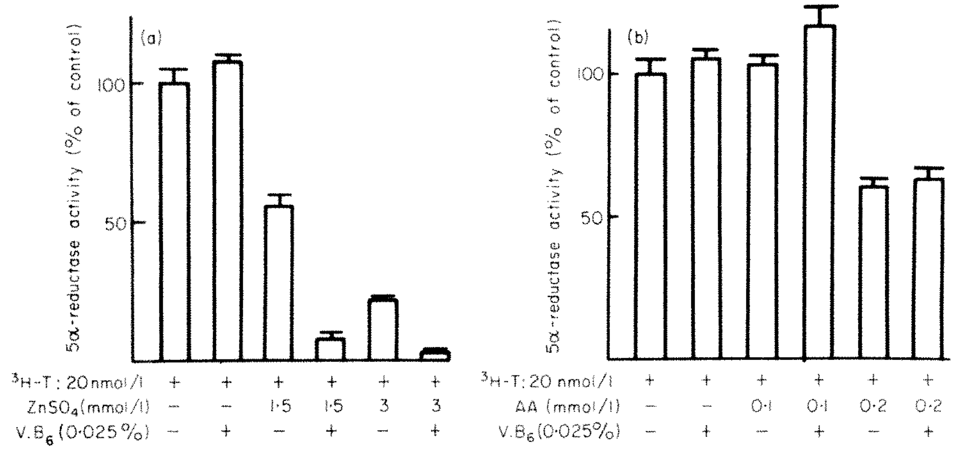

FIGURE 2. Effect of vitamin B6 (VB6) on (a) zinc sulphate and (b) azelaic acid (AA) inhibition of 5α-reductase activity in human skin homogenates. Results are expressed as percentages of controls without inhibitor. Values are means ± SD ($n = 5$). [^{3}H]-T = ^{3}H-testosterone.

Effect of simultaneous addition of zinc, azelaic and vitamin B6 at low concentrations

The additive effect of the three compounds was studied in order to determine minimal concentrations which would effectively inhibit 5α-reductase activity. From the present results, the concentrations used (0·025% vitamin B6, 0·5 mmol/l ZnSO$_4$ and 0·1 mmol/l azelaic acid) were expected to have a minimal effect, or no effect at all, on the enzyme activity. Vitamin B6 0·025% and azelaic acid at 0·1 mmol/l had no effect on the enzyme activity while ZnSO$_4$ at 0·5 mmol/l gave less than 30% inhibition (Fig. 3). In contrast, when all three compounds were added together at these concentrations, 90% inhibition of 5α-reductase activity is observed (Fig. 3).

DISCUSSION

Several previous studies have established the inhibitory action of zinc on the 5α-reductase of human prostate.[7,12,13] We have shown previously that zinc has an inhibitory effect on 5α-reductase in human skin.[8] The present study confirms this effect. In addition, since topical azelaic acid has been reported to have beneficial effects in acne vulgaris,[14] we studied the effects of azelaic acid on 5α-reductase activity. The results have shown that azelaic acid is a potent *in vitro* inhibitor of this enzyme in skin homogenates.

The use of *in vitro* assays to evaluate the local anti-androgenic action of potential 5α-reductase inhibitors has been reported previously in the study of the inhibitory effect of progesterone.[15,16] The main interest of these studies is that they enable the distinction to be made between a local and a systemic effect of the inhibitor. They are of particular value when the inhibitor can be applied topically, as is the case with zinc and azelaic acid, and therefore is less likely to exert systemic effects.

The purpose of the present study was to investigate 5α-reductase inhibition with a view to eventual application in physiological or pathological situations. As plasma testosterone levels vary from 2 nmol/l (women) to 20 nmol/l (men), it appeared pertinent to study zinc and azelaic acid inhibition of 5α-reductase activity at this range of substrate concentrations under conditions which allow a precise measurement of enzyme kinetics.

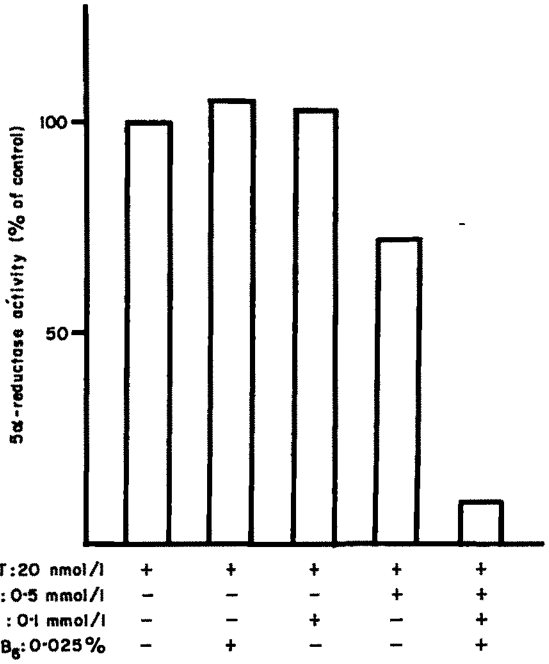

FIGURE 3. Effect of zinc sulphate, azelaic acid (AA) and vitamin B6 (VB6) alone and in combination on 5α-reductase activity in human skin homogenates. Results are expressed as percentages of controls without inhibitor. Values are means of duplicate determinations. [^{3}H]-T = ^{3}H-testosterone.

We have demonstrated a very large inhibition of 5α-reductase activity in the presence of $ZnSO_4$ at concentrations of 3 to 15 mmol/l; 98% inhibition was obtained with the highest concentration. In the human prostate, physiological concentrations of zinc are about 0·2 mmol/l and no correlation has been found between zinc concentration and 5α-reductase activity.[17] However, a biphasic effect of zinc on 5α-reductase activity in the human prostate has been reported, with potentiation at low concentrations (0·1 μmol/l) and inhibition at higher concentrations (3 to 300 mmol/l).[7] This inhibition was shown to be non-competitive relative to testosterone, but competitive relative to NADPH formation. Our preliminary studies (data not shown) seemed to indicate that zinc at low concentrations (0·5 to 3 mmol/l) competitively inhibits 5α-reductase activity while at higher concentrations (3 to 15 mmol/l) it acts as a non-competitive inhibitor. These results suggest that zinc at different concentrations may act by different mechanisms and that this metal ion interferes with different enzymes, since it also inhibits NADP reduction.

Dicarboxylic acids containing 8 to 13 carbon atoms undergo β-oxidation and have been shown to be potent inhibitors of oxydoreductases. It has been proposed that azelaic acid could competitively occupy the NADPH-binding site of 5α-reductase thus resulting in inhibition of the enzyme.[14] In our experiments, azelaic acid was a potent inhibitor of 5α-reductase activity. When zinc and azelaic acid were added together, the effect of these two inhibitors was additive suggesting that they may act by two different mechanisms.

Pyridoxin (vitamin B6) is known to interfere with fat metabolism in the skin and, therefore, to play a role, like the androgens, in the regulation of sebum excretion.[18] It improves acne lesions in adolescents[19] and is more active on topical than on systemic administration.[18] This led us to examine the combined effects of vitamin B6 and zinc. Interestingly, whereas vitamin B6 alone had no effect on 5α-reductase activity of human skin it strongly potentiated the inhibitory effect on zinc. In contrast, vitamin B6 did not potentiate the inhibition of 5α-reductase by azelaic acid. This further supports the hypothesis that zinc and azelaic acid act by two different mechanisms.

When the three substances were tested together, 90% inhibition of the enzyme was obtained at very low concentrations which barely had any effect when tested separately.

If this inhibition is confirmed *in vivo*, a combination of these substances could provide an effective topical treatment for androgen related pathology of human skin.

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Systemic lupus erythematosus with complement deficiency and IgA anti-cardiolipin antibody

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SUMMARY

A case of aggressive systemic lupus erythematosus with a fatal outcome is described. The patient had a complement component deficiency with a strong family history of autoimmune disease. An exceptional feature of the case was the detection of a high titre of anti-cardiolipin antibody of the IgA class only.

Systemic lupus erythematosus (SLE) is a multisystem disease of unknown aetiology and pathogenesis, characterized by the presence of auto-antibodies with tissue injury to many organs. In recent years, analysis of the anti-nuclear antibodies produced by patients with a wide range of auto-immune diseases has allowed a more precise separation of SLE from other disorders such as polymyositis, Sjögren's syndrome and scleroderma, which may share some of the clinical features manifested in lupus patients.[1] Similar studies have helped in identifying clinical subsets within lupus, which may have important pathogenetic and prognostic implications; examples include anti-nuclear factor (ANF) negative lupus, the increased incidence of antibody to the extractable nuclear antigen Ro in chronic cutaneous lupus and the role of anti-Ro antibodies in congenital heart block in neonates born to mothers with SLE.

Such connections between anti-nuclear antibody specificity and disease, although helpful, may prove to be of lesser importance than a recently described group of antibodies directed against phospholipid antigens[2] present in many tissues such as endothelial cell membranes, platelets and neuronal tissue. Two such antibodies, an anti-cardiolipin and an anti-phospholipid 'lupus anticoagulant' are frequently found in patients with SLE and appear to be associated with recurrent thrombosis, foetal loss and thrombocytopaenia.

A high incidence of SLE in patients with complement component deficiencies is well established.[3] It is a paradox that activation of an intact complement cascade mediates much of the tissue injury in SLE, but that patients with complement deficiencies, who are, therefore, unable to fully activate their complement pathways, should develop SLE with an increased frequency.

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We report here a patient who had not only a complement deficiency, but also a high titre of an extremely unusual (immunological class IgA) anti-cardiolipin antibody.

CASE REPORT

A 47-year-old Caucasian female presented with a 6-month history of an urticarial rash involving the neck, cheeks and periorbital regions associated with the sudden onset of Raynaud's phenomenon. Both her brother and sister had sero-positive rheumatoid arthritis, the brother in addition having widespread vasculitis. Her daughter suffered from Raynaud's phenomenon. Examination revealed small, scattered urticarial weals on a background of widespread xerosis with erythematous lesions on the face and neck. The rash partially responded to emollients, topical corticosteroids and systemic antihistamines.

Three months after initial presentation she developed acute right sided pleuritic chest pain, dysphasia and confusion. On examination she was febrile and disorientated, but peripheral neurological examination was normal. An erythematous facial rash, maximal over the malar region was noted. Investigations revealed a normochromic, normocytic anaemia (haemoglobin 9·3 g/dl), leukopaenia, thrombocytopaenia and an elevated erythrocyte sedimentation rate (60 mm/h). Biochemical examination of the urine revealed a significant growth of *E. coli*, but repeated blood cultures were negative. Anti-nuclear factor was positive, although repeated testing for antibodies to double stranded DNA was negative. A cranial scan and cerebrospinal fluid analysis following lumbar puncture were normal. Complement studies revealed depressed levels of C_3 ($<20\%$) and C_4 ($<20\%$) (normal 50–150%), and CH_{50} was undetectable. Clotting studies were positive for the presence of the lupus anticoagulant. A high titre of anti-cardiolipin antibody of the IgA class was noted; no IgG or IgM anti-cardiolipin antibodies were detected. A positive WR was noted, but fluorescent treponemal antibody (FTA) testing was negative, suggesting a false positive reaction. Skin biopsy revealed IgG deposition at the basement membrane zone.

Treatment for presumptive SLE was instituted with oral prednisolone (60 mg/day) and parenteral antibiotics given for her urinary tract infection. However, her mental state deteriorated further. The development of an extensive petechial rash on the extremities heralded the development of a severe coagulation disorder which prevented a renal biopsy being performed. The addition of azathioprine and repeated plasma exchanges were unsuccessful in arresting her disease and she died in respiratory failure 5 weeks after admission to hospital.

At autopsy, a small pericardial and a moderate pleural effusion were noted with areas of congestion and haemorrhage throughout the lung parenchyma and kidneys. Small vessel occlusion with fibrin and platelets was noted in the lungs, liver, myocardium, spleen and kidneys. Immunoglobulin deposition on the glomerulo-capillary walls in the mesangial regions of the glomeruli, the basement membranes of the tubules and Bowmans capsules was also noted, strongly suggestive of lupus nephritis. There was no evidence of infection. It was felt that disseminated intravascular coagulation had developed on a background of SLE, with death a direct result of respiratory failure.

DISCUSSION

As no single clinical sign or laboratory investigation is specific to systemic lupus erythematosus, the diagnosis is based on a combination of clinical and pathological findings, each individually being found in other auto-immune conditions, but when taken together suggesting a diagnosis

TABLE 1. Presence in our patient of revised American Rheumatism Association criteria (1982) for diagnosis of SLE

Malar rash	+
Discoid rash	–
Photosensitivity	–
Oral ulceration	–
Non-erosive arthritis	–
Serositis	+
Renal disorder	–
Neurological disorder	–
Haematological disorder	+
Immunological disorder	+
Anti-nuclear antibody	+

of SLE. The preliminary criteria for diagnosis of SLE published by the American Rheumatism Association (ARA) in 1971[4] did not incorporate serological tests now used in the diagnosis and management of SLE, such as antibodies to native DNA and Sm nuclear antigen or anti-nuclear factor testing. Therefore, in 1982, revised criteria were published by the ARA,[5] incorporating these more recent serological investigations, which have proved so useful in distinguishing patients suffering from SLE from those with other disease such as dermato or polymyositis and scleroderma.

The 1982 revised ARA criteria present in our patient are summarized in Table 1. Four out of the 11 criteria must be present in a patient serially or simultaneously during observation, for a diagnosis of SLE to be made. A firm diagnosis of SLE was established in our patient, based on the clinical findings of a malar rash and evidence of pleuritic disease (chest pain and effusion). Laboratory investigations revealed anaemia, leukopaenia and thrombocytopaenia (counted as one criterion under haematological disorder), a biological-false positive test for syphilis (positive immunological disorder) and a positive anti-nuclear factor. Whilst our patient developed acute dysphasia and slurred speech, the neurological criteria of the ARA classification comprise either seizure or psychosis, neither of which was present in our patient, and this criterion was not satisfied, therefore.

Considerable interest has recently developed concerning a group of antibodies which occur frequently in patients with SLE and are directed against phospholipid antigens. Two such antibodies are the 'lupus anticoagulant' and an anti-cardiolipin (a-CL); both of which were present in our patient. The presence of an anticoagulant in the blood of lupus patients, detected by prolongation of clotting studies, was first described in 1952[6] and was later characterized as an anti-phospholipid antibody.[7] The lupus anticoagulant appears to act at the level of the prothrombin activator complex in the clotting cascade, prolonging all phospholipid dependent coagulation tests, but paradoxically, in clinical practice is associated with an increased tendency to thrombosis, not bleeding.[8]

There are many similarities between a-CL antibodies and the lupus anticoagulant, both antibodies being directed against phospholipid antigens which are widely distributed throughout tissues of the body such as endothelial cell membranes, platelets and neuronal cells.[9] Anti-cardiolipin antibodies were initially associated with the development of recurrent thrombosis,[2] thrombocytopaenia[10] and foetal loss[11] in SLE. More recently a-CL antibodies have been associated with a wide range of clinical features such as labile hypertension, livedo reticularis and arterial occlusions, often in patients who do not have anti-nuclear antibodies and may hence fail to fulfil the ARA criteria for SLE. This group of patients are often described as having 'lupus-like' disease, and more recently the term 'anti-cardiolipin/phospholipid syndrome' has been proposed.[12] The relationship of livedo reticularis to cerebrovascular disease and hypertension was first noted in 1965 by Sneddon[13] and is now known as Sneddon's syndrome. In view of the striking clinical features shared by patients with the anti-phospholipid and Sneddon syndromes, it is interesting to speculate on the relationship between these two conditions. Whilst the exact mechanism of action of phospholipid antibodies is not known, it is thought that they may bind to endothelial cells and impair arachidonic acid release, thereby reducing prostacyclin production allowing platelet aggregation. Alternatively, inhibition of prekallikrein activity seems to occur, which impairs fibrin clearance. Finally, direct binding to platelet phospholipid may cause platelet damage increasing their adhesiveness and initiating thrombosis.[14]

The finding in our patient of a high titre of a-CL antibody of the IgA class without detection of an IgG or IgM a-CL is extremely unusual. In a study of 40 patients with elevated a-CL antibodies associated with either thrombosis, thrombocytopaenia or recurrent foetal loss,[15] only one patient had elevated IgA a-CL alone, the majority having raised IgG or IgM a-CL antibodies with or without elevated IgA a-CL antibody. The implications of this class specific response are not clear, but it is of interest that in patients with acute Guillain-Barré syndrome reported recently,[16] particularly high levels of IgA a-CL antibodies correlated with severe disease requiring ventilation, or with a fatal outcome. This may imply a pathogenetic role for these antibodies in this condition, with an IgA response to neuronal phospholipid.

The standard tests for syphilis detect the antiphospholipid antibody reagin by agglutination (VDRL) and complement fixation tests (Wasserman reaction). As was the case in our patient, up to 30% of patients with the lupus anticoagulant or a-CL antibodies have a biological false positive test for syphilis, but this is usually at a low titre[10] and is too insensitive to use as a screening procedure for anti-phospholipid antibodies.

The complement system plays a central role in mediating tissue injury in immune complex diseases such as SLE. It is, therefore, surprising that genetic deficiencies of complement components should predispose the individual to develop auto-immune disease. There is a particularly high incidence of SLE in patients with deficiencies of the early components of the classical pathway such as C_1, C_4 and C_2.[3] The complement studies in our patient were strongly suggestive of an inherited complement deficiency; whilst most patients with SLE have decreased complement levels at some time in their disease, patients with inherited complement deficiency SLE (CD-SLE) are easily differentiated.[17] In both active SLE and CD-SLE, levels of C_3, C_4 and CH_{50}, (a measure of overall complement activity, mainly of the classical pathway) are reduced. However, in CD-SLE the reduction in CH_{50} is out of proportion to the reduction in the other components, and, as in our patient, CH_{50} is usually undetectable. During active disease in non CD-SLE, CH_{50} is always measurable, usually greater than 5% of the normal value. The mechanism by which complement deficiency predisposes to auto-immunity is unknown, but may be a result of a failure properly to solubilize immune complexes in the blood (for which fixation of complexes with C_3 and C_4 is necessary).[18] Alternatively, inadequate binding of immune complexes to erythrocytes via a complement receptor may cause complexes to marginate in the plasma component of blood, leading to contact and phagocytosis by endothelial cells with the initiation of local inflammation.[19] It is likely that incomplete, heterozygous deficiencies of complement are not infrequent, and these may account for a substantial number of cases of SLE.[20] Complement studies on other members of our patient's family had only been possible on the daughter with Raynaud's phenomenon, at the time of writing, and showed a normal profile. Testing of the brother and sister with significant connective tissue disease may well reveal abnormalities. As is characteristic of CD-SLE, our patient produced no antibodies to ds-DNA, these antibodies being present in only 25% of such SLE patients.[17]

The aggressive clinical progression in our patient was not typical of CD-SLE which often follows a relatively benign course. However, in view of the CNS involvement and widespread coagulopathy, it is interesting to speculate on the role of the IgA a-CL antibody which has already been shown to carry a poor prognosis in acute Guillain-Barré syndrome. We are unaware of any previous association of SLE, complement deficiency and IgA a-CL antibody.

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Plasma somatomedin-C levels in systemic sclerosis

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SUMMARY

We have measured the plasma levels of somatomedin-C (SM-C) or insulin-like growth factor 1 (IGF-I) in 13 patients with progressive systemic sclerosis (PSS) and age and sex matched healthy controls. We found the plasma SM-C levels to be within normal limits in all the patients. Thus, if somatomedin-C plays a role in the pathogenesis of PSS, it is more likely to be at the fibroblast receptor level or in the synthetic response of fibroblasts to SM-C.

Progressive systemic sclerosis (PSS) is characterized by Raynaud's phenomenon, skin thickening, and internal organ involvement due to increased collagen deposition. The pathogenesis of PSS is unknown, but is believed to involve an interplay of vascular damage, immunological abnormalities and increased fibroblast synthetic activity.[1] One hypothesis is that the exposure of PSS fibroblasts to growth factors or other cytokines causes increased mitogenic and synthetic activity.[2,3]

Purified growth factors have been tested for possible selective effects on PSS fibroblast cultures. LeRoy *et al.*[2] found that platelet-derived growth factor (PDGF) had a selective mitogenic effect on normal fibroblasts, but not on PSS fibroblasts in culture. Falanga *et al.*[3] demonstrated a selective increase in glycosaminoglycan (GAG) synthesis by cultures of PSS fibroblasts compared with normal fibroblasts in response to transforming growth factor-beta (TGF-beta). However, it is unclear how these *in vitro* observations relate to the pathogenesis of PSS. Botstein *et al.*[4] have proposed that a clonal selection of fibroblasts with excessive synthetic activity plays a role in PSS. This attractive hypothesis would require an enhanced proliferation of those fibroblasts having a greater synthetic activity.

A mitogenic response to a growth factor requires the presence of a progression factor—a factor which permits cells to complete the G1 phase of the cell cycle and begin DNA synthesis.[5] Neither PDGF nor TGF-beta is a progression factor, but somatomedin-C (SM-C) is. SM-C or insulin-like growth factor 1 (IGF-1) is produced primarily in the liver and is best known as the polypeptide hormone which mediates the cartilage stimulating activity of growth hormone.[6] In

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addition to being a progression factor for fibroblast mitogenesis, SM-C may increase the formation of connective tissue.[7]

We are interested in the role of SM-C in disease processes, since somatostatin analogues that interfere with the synthesis of SM-C are now becoming available.[8] We believe that if plasma SM-C levels are elevated in PSS, the vascular damage present in this disease would permit even greater exposure of fibroblasts to SM-C. Thus, the action of SM-C alone, or in combination with other growth factors, might stimulate excessive connective tissue synthesis. We, therefore, measured plasma SM-C levels in patients with various clinical stages of PSS.

METHODS

Subjects

Thirteen patients with PSS (two males, 11 females; mean age 51 years, range 31–70) participated in the study. None of the patients were receiving drugs known to affect connective tissue synthesis, such as D-penicillamine or colchicine. One patient with myositis was receiving prednisone and one was on azathioprine. Patients were classified according to extent of cutaneous involvement as having diffuse disease, or the limited acrosclerotic form of the disease. Active disease was defined as the progression of cutaneous or systemic involvement.

Somatomedin-C levels

Plasma SM-C levels were measured by an acid alcohol extraction technique followed by radioimmunoassay.[9] In this technique, the patient's plasma is incubated with 87.5% ethanol and 0·125 M HCl with subsequent centrifugation at 1860 *g* for 30 h at 4°C, followed by incubation with 0·855 M Tris base (pH 7·4) for 20 h and standard double antibody

TABLE 1. Clinical features and plasma somatomedin-C levels in patients with progressive systemic sclerosis

	Patient no.	Active disease	Age (years)	Sex	Internal organ involvement				Plasma SM-C (ng/ml)
					Oesophagus	Lung	Heart	Kidney	
Diffuse disease	1	+	31	F	+	–	–	–	248
	2	+	39	F	–	–	–	–	225
	3	+	52	F	+	–	–	–	125
	4	–	38	M	–	–	–	–	150
	5	–	51	F	+	–	–	–	209
	6	–	60	M	+	–	–	–	225
	7	–	65	F	+	–	–	–	117
Limited disease	8	+	36	F	–	–	–	+	198
	9	+	43	F	–	+	–	–	201
	10	–	52	F	+	–	+	–	128
	11	–	60	F	–	–	–	–	128
	12	–	63	F	–	–	+	–	98
	13	–	70	F	+	–	–	–	174

Plasma SM-C levels in 13 age and sex matched healthy controls 51-330 ng/ml.

radioimmunoassay. The patients' plasma levels of SM-C were compared with the levels in age and sex matched healthy controls. Student's t-test was used for statistical analysis.

RESULTS

The clinical features and plasma SM-C levels in our patients are shown in Table 1. Disease duration was greater than 3 years in eight patients (61%) and 3 years or less in five patients (39%). Raynaud's phenomenon was present in nine patients (69%). Systemic involvement included gastrointestinal disease in eight patients (62%), pulmonary disease in one (8%), cardiac involvement in two (15%), renal disease in one (8%), and myositis in two (15%).

The mean plasma SM-C level (± SD) in the PSS patients was 175 ± 55 ng/ml (range 65–284 ng/ml). This was within the normal range found in the controls, 51–330 ng/ml. The seven patients with diffuse disease had a mean plasma SM-C level of 192 ± 61 ng/ml compared with 154 ± 42 ng/ml in the 6 patients with limited disease, but the difference was not statistically significant. Plasma SM-C levels in the patients with active disease were not significantly different from those in patients with inactive disease.

DISCUSSION

We have shown that plasma levels of somatomedin-C, an important progression factor for other growth factors, are normal in patients with PSS. SM-C or IGF-1 is a 7,649 mol. wt. 70 amino acid polypeptide with significant homology to human proinsulin. SM-C is produced primarily in the liver in response to growth hormone and is non-covalently bound to specific carrier proteins present in plasma. In addition to regulation by growth hormone, SM-C plasma levels are affected by a number of factors including nutritional status, oestrogen and thyroxine levels, and hepatic and renal disease. The target tissue effects of SM-C include cartilage stimulating activity, insulin-like activity in adipocytes and muscle cells, mitogenic activity in fibroblasts, and stimulation of collagen synthesis by human dermal fibroblasts.[6,7]

This is the first time, to our knowledge, that plasma levels of a growth factor have been measured in patients with PSS, although the response of PSS fibroblasts to growth factors has been investigated previously. LeRoy *et al.*[2] demonstrated that platelet derived growth factor (PDGF), epidermal growth factor (EGF), nerve growth factor, and fibroblast growth factor did not selectively enhance collagen synthesis independently of replication in either PSS or normal fibroblasts. However, PDGF had a selective mitogenic effect on control fibroblasts, when compared with PSS fibroblasts.

More recent work by Falanga *et al.*[3] has shown that TGF-beta preferentially increases GAG synthesis by cultures of PSS fibroblasts compared with normal fibroblasts. These previous investigations have started to focus more attention on the role of growth factors in disease processes, particularly those manifested by fibrosis. Recently, Martinet *et al.*[10] have shown that macrophages isolated from the lungs of patients with pulmonary fibrosis secrete more PDGF than those from normal lungs. Thus, the concept of fibrosis, either because of an inherently increased response of target cells or because of an abnormal synthesis of growth factors, is gaining momentum.

In work later confirmed by others,[11] LeRoy *et al.*[12] showed that dermal fibroblasts isolated from patients with PSS produce increased amounts of collagen and other matrix proteins. Since these cultures continue to behave abnormally through multiple *in vitro* passages, it has been hypothesized that a clonal selection of fibroblasts with greater synthetic activity is operating in

PSS.[4] The existence of subsets of fibroblasts with different rates of collagen synthesis has already been shown.[13] There are two mechanisms by which growth factors promote fibroblast mitogenesis. PDGF, for example, acts as a competence factor to help initiate the G1 phase of the cell cycle.[10] Conversely somatomedins and EGF act as progression factors to permit competent cells to complete G1 and begin DNA synthesis.[5] Thus, SM-C may play an important role in acting synergistically with other growth factors to promote fibroblast replication.

One possible mechanism of fibroblast selection and stimulation by growth factors in PSS is the vascular damage that appears to occur early in the disease process. Vascular abnormalities in PSS include perivascular fibrosis, intimal proliferation, and damaged endothelial cells. Ultrastructurally, the endothelial cells show separation of tight junctions, vacuolization, oedema, granular degeneration of the nucleus, and necrosis.[14] Such endothelial cell damage may lead to the leakage of excessive amounts of plasma growth factors, including SM-C, into the tissues, and thus promote PSS fibroblast proliferation and synthetic activity. Moreover, damaged endothelium may lead to further activation of platelets and release of their growth factors.

In the present study, we found that plasma SM-C levels were normal in patients with PSS, regardless of the extent or activity of their disease. These findings do not exclude, however, the possibility of increased tissue levels of SM-C in PSS, or an altered responsiveness of PSS fibroblasts to this progression growth factor.

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Hand warming as a treatment for Raynaud's phenomenon in systemic sclerosis

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SUMMARY

Twelve patients with Raynaud's phenomenon (RP) due to systemic sclerosis (SS) warmed their hands for 5 min in hand hot water every 4 h throughout the day during alternate weeks of a 6-week study. There was a statistically significant decrease in the number and duration of Raynaud's attacks in the weeks in which warming was performed compared with the intervening weeks. An increase in blood flow as measured by laser-Doppler flowmetry accompanied clinical improvement. Simple hand warming appears to be effective in the management of RP in patients with SS.

Ninety five per cent of patients with systemic sclerosis (SS) suffer from Raynaud's phenomenon (RP) at some stage of their illness.[1] Many vasodilatory drugs have been used to treat this condition. These include reserpine,[2] guanethidine,[3] and most recently the calcium channel blocking drug, nifedipine.[4,5] All of these agents produce some symptomatic improvement, though less in SS than in primary RP.[6] However, clinical improvement is associated with little improvement in blood flow,[5] possibly because all of these agents produce a fall in blood pressure, and there is evidence that finger blood flow in SS may be primarily determined by the arterial pressure.[7] We have recently demonstrated the vasodilatory effect of simple hand warming in patients SS,[8] and present here the results of an open investigation of the effect of simple hand warming on the frequency and duration of RP in 12 patients with SS.

METHODS

During the winter months of 1986–1987, 12 patients with RP due to SS, who had been demonstrated to show vasodilation for up to 2 h in response to warming of both hands in water at 35°C, gave their informed consent to taking part in a trial of prophylactic hand warming. The trial was carried out over a 6-week period. A symptom diary was kept by each patient, recording the frequency and duration of attacks, and how often they were able to go out of the house. The

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study commenced with patients recording symptoms during a week in which they followed their normal routine (week 1). In the second week of the study, patients warmed their hands by immersing them in hand hot water for 5 min every 4 h. This warming procedure was also carried out in weeks 4 and 6 of the study, and in weeks 3 and 5 patients did not warm their hands in warm water. At the end of 6 weeks, patients were asked to state whether they had benefitted, clinically, during periods of warming compared with periods of non-warming. Finger blood flow was measured by laser-Doppler flowmetry in eight patients at the start of the study and at the end of the final period of warming. All measurements were carried out in a constant temperature environment (ambient temperature 28°C).

Statistical analysis of the number and duration of attacks, and of the number of times that the patient went outside (all obtained from the patient's symptom diary), was by Wilcoxon's signed rank test, and McNemar's test was used for the patient preferences.

RESULTS

The results are presented in Table 1. There was a statistically significant reduction both in the number ($P < 0\cdot01$) and duration ($P < 0\cdot05$) of attacks of RP during periods of warming compared with periods of non-warming. Eleven of the 12 patients preferred the periods of warming, whilst one was unable to decide between the two alternatives ($P < 0\cdot01$). In addition, patients recorded going outside more frequently during periods of warming, but this difference was not statistically significant. The weather was uniformly cold throughout the study period and was unlikely to have influenced the results in either direction. Laser-Doppler blood flow increased significantly in all eight patients in whom it was measured ($P < 0\cdot01$) (Fig. 1).

TABLE 1. Number and duration of attacks of RP in 12 patients with systemic sclerosis treated by simple hand warming

Patient no.	No. of attacks/week		Mean duration of attacks (min)		No. of times outside/week	
	warmed	untreated	warmed	untreated	warmed	untreated
1	5	5	11	13	4	4
2	16	20	44	50	26	7
3	25	21	17	18	7	12
4	2	7	20	20	27	33
5	21	23	12	19	20	12
6	14	16	43	52	13	20
7	12	18	36	42	22	17
8	7	11	40	41	15	12
9	7	10	55	55	13	11
10	3	8	8	10	3	1
11	8	10	9	26	4	2
12	22	24	14	17	15	20
mean	11·8**	14·4	26*	30	14	12·5

Significantly less than during untreated periods $^{*}P < 0\cdot05$, $^{**}P < 0\cdot01$.

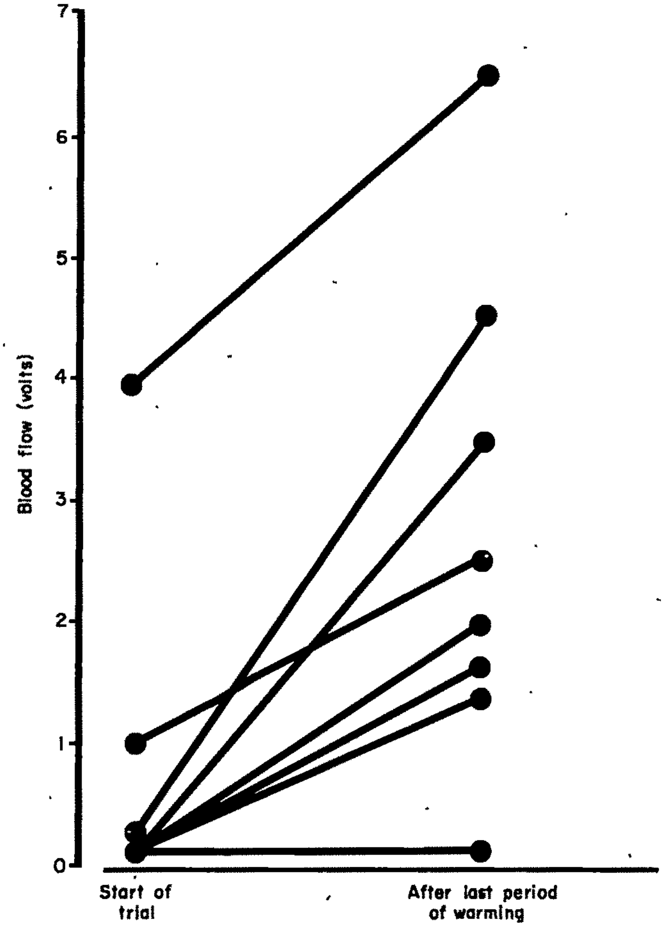

FIGURE 1. Laser-Doppler blood flow in arbitrary units (volts) recorded before the study and after the last period of warming in eight patients with SS.

DISCUSSION

Warming as treatment for RP has been used previously as part of a biofeedback experiment in which patients were exposed to a cold ambient temperature whilst warming the hands in warm water.[9,10] This produced improvement in symptoms and an increase in skin temperature. Our results suggest that it is not necessary to perform the warming procedure in the cold to achieve benefit, since simple warming alone is sufficient to give prolonged vasodilatation and clinical improvement. The present study shows that such treatment is valuable, reducing the number and duration of attacks to a degree that is comparable to the reported effect of treatment with oral vasodilators,[3-5] without the risk of side effects which many patients develop at therapeutic dosages. Although the mean improvement does not appear to be large, there was considerable improvement in some patients, and little change in some others—it was impossible to predict on clinical grounds which patients would do well. Patients expressed their approval of this simple treatment, despite the relatively small overall change in the numbers and duration of Raynaud's attacks. While this may represent a placebo effect, patient approval probably was related to a co-existent improvement in the texture of the skin, upon which many patients spontaneously commented and which is an important additional benefit. The clinical improvement was accompanied by measurable changes in blood flow, which again appear to be comparable to those reported with vasodilating agents.[5]

It seems that simple warming procedures are effective in the treatment of RP in SS. Our patients are now advised to warm their hands by immersion in warm water before going out of doors. This is more practical than warming every 4 h by the clock. The duration of the effect of such warming on symptoms and blood flow varies between patients, but is never less than 2 h.[8] In addition, they should be generally warm before entering a cold environment, a precaution that seems sensible and likely to give further benefit. Moreover, the use of electrically heated gloves (Vascutherm®, available under the NHS), keeps the hands warm. Several of our patients have decided to make use of other warming devices (hot water bottles or chemical hand warmers) to keep their hands warm whilst travelling to work. All have received nifedipine in the past, without great benefit, and all are very clear in their preference for simple non-pharmacological therapy. Temperature changes are known to influence connective tissue viscosity,[11] and perhaps this explains the benefit to skin texture on which patients have spontaneously commented. Temperature also influences blood viscosity and may also partly explain the improvement in blood flow. It has been suggested that a decrease in connective tissue viscosity allows greater dilatation of blood vessels, but this is speculative. Whatever the precise mechanism, hand warming in warm water seems a useful addition to our available therapy for what is sometimes an intractable problem.

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In vitro transepidermal water loss: differences between black and white human skin

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SUMMARY

In vivo transepidermal water loss studies are characterized by large inter-individual variability and biased by environmental effects and eccrine sweating. An *in vitro* technique for measuring transepidermal water loss (TEWL) was used to compare TEWL in two racial groups—blacks and whites. In both groups a significant correlation between skin temperature and increased TEWL was found ($P < 0{\cdot}01$). Furthermore, black skin had a significantly higher mean TEWL than white skin, corrected log TEWL 2·79 and 2·61 $\mu g/cm^2/h$, respectively. The difference in TEWL between the groups could be because measurements were made in the absence of eccrine sweating and other vital functions. The higher TEWL in black skin could be explained on the basis of a thermoregulatory mechanism. The anatomical and physiological differences in the stratum corneum between different races are discussed.

Few publications compare the different functional properties of black and white human skin. Previous studies have revealed some differences in stratum corneum structure and composition. Weigand *et al.*[1] measured the anatomophysical properties of black and white skin, and observed that black stratum corneum was more compact. In the present study we have measured *in vitro* water loss from black and white human skin to explore the possible physiological significance of Weigand's observations.

METHODS

Subjects

Full thickness skin samples were obtained from refrigerated cadavers, 12 Caucasian and 10 black (age range 5–72 years) with appropriate permits. Black subjects were all Americans of Afro-Carribean ethnic origin. Subjects in the two groups were age- and sex-matched (Table 1).

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TABLE 1. Details of subjects sampled for the study

Black		Caucasian	
Age (years)	Sex	Age (years)	Sex
5	F	10	F
17	M	15	M
23	M	29	M
32	M	32	M
37	M	33	M
42	F	38	F
45	F	47	F
48	M	47	M
65	M	53	F
72	M	59	M
		65	M
		66	M
Mean 38·6		41·1	

Two samples were taken from adjacent inner thigh sites and refrigerated for 12–24 h before measurement.

Experimental procedure

Two skin samples, 1·8 cm diameter, trimmed of subcutaneous fat, were acclimated to ambient conditions for 2 h, epidermis up, in a Petri dish containing cottom gauze saturated with Hank's solution.

Following acclimatization, one sample was placed dermis down over the mouth of a glass chamber (1·8 cm diameter; ACE glass Inc., Vineland, NJ. U.S.A.) containing Hank's solution, and closed with a pressure releasing stopper. An 'O' ring was placed on the stratum corneum side of the sample and then an evaporation chamber (#28, Thomas, Philadelphia, PA, U.S.A.) was clamped in place forming a water-tight seal. The top of the evaporation chamber was closed with a modified rubber stopper containing inflow and outflow ports.

The chamber was mounted on an adjustable arm. The inflow port was connected to a cylinder of pressurized nitrogen (99·9%) with plastic tubing and the outflow port was connected to a Meeco Manufacturers Engineering and Equipment Corporation, Warrington, PA, U.S.A.) model W electrolytic water analyser. The nitrogen flow rate through the chamber was adjusted to 100 ml/min and the gas contained 30–35 ppm water vapour. The skin sample was acclimatized at room temperature until the exponential decrease in evaporation due to skin surface drying dropped to 0·5 ppm/min, or less (approximately 45 min). This operation represented stabilization; total stabilization under dry nitrogen required several more hours.

Once stabilized, the chamber was immersed sequentially in each of five 2000 ml water baths agitated with air bubbles and maintained at 20, 25, 30, 35 and 40°C, respectively with aquarium heaters (Supreme, Heatmaster, E.G. Danner MFG Inc. Central Islip, NJ, U.S.A.) accurate to ±0·5°C. The chamber was arranged with the skin sample parallel to the water surface and the section of the chamber containing the Hank's solution immersed. Aeration splash on the chamber was blocked with a piece of Parafilm. Evaporation rate was recorded first in the 20°C bath; once stabilized, the chamber then was immersed in the next water bath (25°C) and the measurements repeated. This was repeated in each of the remaining baths in order of increasing temperature. Bath temperature was monitored near the chamber with a tele-thermometer probe (Yellow Springs Instrument Company, Ohio, U.S.A.). The amount of water vapour in the nitrogen gas was recorded by the Meeco analyser in parts in per million (ppm).

Water evaporation was converted to transepidermal water loss (TEWL) ($\mu g/cm^2/h$) using the equation:

$$\text{TEWL} = \text{Eppm}\ (0{\cdot}0736\ \mu g/\text{min})(60\ \text{min})/2{\cdot}545\ \text{cm}^2$$

where: Eppm = water evaporation in ppm; 0·0735 μg/min = factor for converting 1 ppm H_2O/100 ml to μg H_2O/ml at 25°C and 760 mm Hg, and 2·545 cm^2 = area of skin sample based on the internal diameter of the 'O' ring.[2]

The second sample of skin from each subject was used to obtain skin temperature measurements. The sample was placed, dermis down, on a glass chamber; a tele-thermometer probe was centered over the stratum corneum and a layer of cotton gauze was placed over the probe and skin. An 'O' ring was centered over the gauze and the other portion of the chamber carefully clamped in place, producing a water-tight seal. The gauze pressed the sensor against the skin surface. The portion of the chamber facing the dermis was filled with Hank's solution and sealed with a pressure releasing stopper. The skin temperature chamber was mounted on a ringstand arm and arranged in a water bath in an identical way to the evaporation chamber. The water bath testing sequence was 40–20°C; skin temperatures stabilized 10 min following immersion. With water bath temperature controlled, the skin temperature recorded was assumed to approximate to that of the skin in the evaporation chamber. Additional experimental details are described by Mathias *et al.*[3]

RESULTS

Skin temperature best approximated that of the stratum corneum and correlated well with bath temperatures (Table 2). The skin–bath temperature difference increased almost linearly as the bath temperature deviated from 25°C. Skin temperatures only were used in calculations.

The TEWL of Caucasian and black skin specimens increased exponentially with skin temperature; substituting log TEWL produced a linear relationship (Fig. 1) in accordance with Grice *et al.*[4] The Caucasian and black log TEWL–skin temperature values were analysed by linear regression analysis. The Caucasian data produced a highly significant correlation ($r = 0{\cdot}828$; $P < 0{\cdot}01$) a slope of 0·034 log TEWL/°C, a y intercept of 1·590 log TEWL and a grand mean point of 28·86°C, 2·58 log TEWL. The blacks produced a highly significant correlation ($r = 0{\cdot}875$; $P < 0{\cdot}01$), a slope of 0·036 log TEWL/°C; the y intercept was 1·730 log TEWL and the grand mean point: 28·86°C, 2·76 log TEWL.

Thus, the increase in TEWL with increase in skin temperature appeared to be very similar in Caucasian and black skin samples.

Since the log TEWL–skin temperature relationships appeared linear and the slopes similar for Caucasians and blacks, TEWL values for a single bath temperature were compared. TEWL variability due to different skin temperatures, found in any bath situation, was reduced by adjusting all TEWL to a common 30°C following Grice *et al.*[4] using the equation:

$$\log \text{TEWL}_{30} = \log \text{TEWL}_{T} + \text{slope}\ (30 - T)$$

where: log TEWL_{30} = log TEWL corrected to 30°C, log TEWL = log TEWL at actual skin

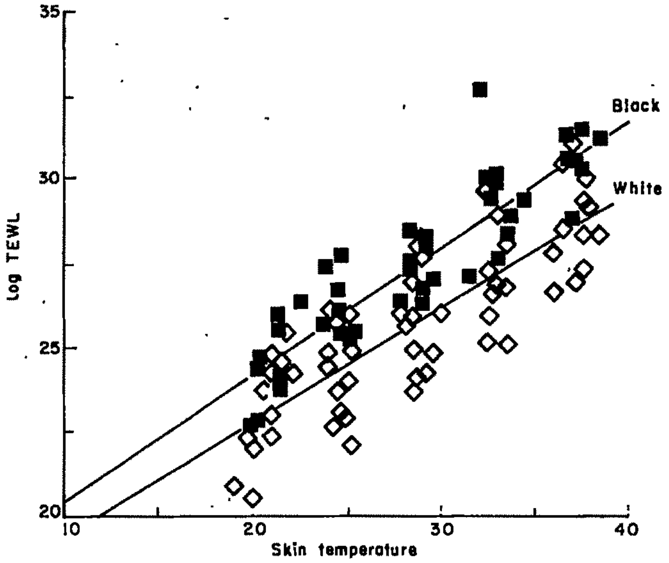

FIGURE 1. Relationship between TEWL and skin temperature in samples of Caucasian and black skin *in vitro*.

TABLE 2. Values for transepidermal water loss (TEWL) from Caucasian and black skin at different temperatures

	Bath temperature °C	Skin temperature °C	TEWL (log)* (mean ± SD)
White	20	20·7	2·64±0·13
	25	24·6	2·60±0·13
	30	28·8	2·61±0·14
	35	33·0	2·61±0·15
	40	37·1	2·62±0·13
			mean 2·61
black	20	21·06	2·75±0·09
	25	24·5	2·80±0·09
	30	29·03	2·76±0·08
	35	33·12	2·83±0·15
	40	37·2	2·81±0·07
			mean 2·79

* Log TEWL corrected to a skin temperature of 30°C (see text). Values significantly different in Caucasian and black skin at all temperatures ($P < 0·01$).

temperature, slope = regression coefficient (log TEWL/°C), and $30-\mathrm{T}$ = difference of actual skin temperature from 30°C. The slopes 0·034 and 0·036 were, respectively, utilized for Caucasians and blacks in the equation. Student's *t*-test was used to compare the two sets of data. The results are shown in Table 2. The adjusted log TEWL values for Caucasians and blacks were significantly different at all temperatures ($P < 0{\cdot}01$).

DISCUSSION

The stratum corneum is of equal thickness in blacks and whites;[5,6] however, Weigand *et al.*[1] demonstrated that the stratum corneum from blacks contained more cell layers and required more tape strips to remove it than that of Caucasians. They found a great deal of variation in the values obtained from black subjects, while data from Caucasians were more homogeneous. No correlation was found between the degree of pigmentation and the number of cell layers. These data could be explained on the basis of greater intercellular cohesion resulting in a increased number of cell layers and an increased resistance to stripping in blacks.

This mechanism may involve lipids since the stratum corneum lipid content studied by Reinertson and Wheately[7] on abdominal skin revealed 8·5–18·4% of lipids with higher values in black subjects. This was confirmed by Weigand *et al*;[1] they showed that delipidized specimens of stratum corneum from both races were equal in weight, while differing in air density and isopyknic sucrose gradient. In accordance with this, Johnson and Corah[8] found mean electrical resistance of skin from adult blacks twice that of skin from adult whites suggesting an increased cohesion or thickness of the stratum corneum. Water absorption by the skin has been studied by Bronaugh *et al.*[9] in an *in vitro* model. No significant differences were recorded between whites and blacks.

In our study, a correlation between temperature and *in vitro* TEWL was found in both blacks and whites. TEWL increased exponentially with skin temperature. Absolute TEWL values were significantly higher in black skin. This data is difficult to interpret in the light of physicochemical differences between black and white skin reported previously; furthermore, the study was peformed *in vitro* where the effects of some physiological functions, such as sweating, are not detectable. The results could be explained on the basis of a different responsiveness of the black skin to thermal stimulation. *In vivo* studies have shown that during exercise blacks have a lower skin and rectal temperature.[10] Assuming a relationship between *in vivo* and *in vitro* experiments we suggest that in maintaining the same skin temperature, a greater TEWL rate in blacks would be expected (if basically *in vivo* TEWL is the same in the two races). Indeed, recently, we observed a higher *in vivo* TEWL in young blacks.[11] The difference was recorded both in untreated skin and in skin exposed to different concentrations of sodium lauryl sulphate. There appears to be different water barrier behaviour in the two races; blacks have a broader range of TEWL responses to sodium lauryl sulphate. Varying concentrations elicit strikingly different responses. Increasing percutaneous penetration with occlusion caused an increased response with the lower irritant concentration, but not with the higher, since the TEWL levels were already high.

The present *in vitro* study confirms higher TEWL values in black skin. This data is scientifically sound compared with our *in vivo* studies, since in this model the influence of sweating was removed and skin temperature kept constant. Much work remains in confirming and extending these data and characterizing other differences between black and white skin. Nevertheless, we are impressed by the concordance between the more variable *in vivo* results and the more rigorous *in vitro* results, indicating an increased water loss in black skin.

Traditionally, black and white skin is believed to vary principally in colour; as more sophisticated questions are asked with contemporary technology, we hopefully will understand their functional, clinical and evolutionary significance.

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Polymorphonuclear leukocyte random migration and chemotaxis in psoriatic and healthy adult smokers and non-smokers

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SUMMARY

Random migration and chemotactic responsiveness of polymorphonuclear leukocytes (PMNs) from psoriatic and control smokers and non-smokers were studied *in vitro* and *in vivo*. *In vivo*, but not *in vitro*, PMNs from psoriatic smokers responded to a significantly greater degree to a standard chemotaxin than did PMNs from psoriatic non-smokers, control smokers and control non-smokers. This suggests that smoking has a selective effect on psoriatic PMNs. The possible relevance of this to the pathogenesis of psoriasis vulgaris and to palmoplantar pustulosis is discussed.

O'Doherty and Macintyre[1] recently reported a multicentre case control study which demonstrated a significantly higher prevalence of smoking in patients with palmoplantar pustulosis (PPP) when compared with healthy controls. A characteristic feature of this chronic condition is the presence of sterile pustules predominantly on the thenar or hypothenar eminences and the sole or sides of the heel. The presence within these pustules of large numbers of polymorphonuclear granulocytes (PMNs) raises the possibility that these cells play a major role in the pathogenesis of this condition.

At present, PPP tends to be considered as a separate, although closely related, entity to psoriasis vulgaris.[2] However, the conditions do have similar histological features, in particular the early appearance and subsequent accumulation of PMNs within and above the psoriatic epidermis.[3,4] Several authors have reported defects of various PMN functions, including mobility, in psoriasis[5–7] prompting suggestions that PMN abnormalities play a fundamental role in the pathogenesis of psoriasis. Similarly, enhancement of PMN chemotaxis demonstrated in patients with PPP was indistinguishable, both quantitatively and qualitatively from that observed in patients with pustular psoriasis, a condition that has features in common with both PPP and psoriasis vulgaris.[8]

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No information is available, to our knowledge, regarding the effect of smoking on PMN function in psoriatic patients or in those with PPP.

In the present study we examined the effect of smoking on psoriatic and control PMN motility *in vitro* and *in vivo*.

METHODS

Subjects

A total of 66 psoriatic smokers (39 female, 27 male; age range 18–71 years, mean 37); 45 psoriatic non-smokers (24 female, 21 male; age range 16–65 years, mean 35); 64 control smokers (38 female, 26 male; age range 18–69 years, mean 36) and 43 control non-smokers (23 female, 20 male; age range 17–68 years, mean 36) took part in the study.

All patients and controls in both the *in vitro* and *in vivo* studies abstained from alcohol during the course of the study and none had any unusual dietary habits. Total and differential white blood cell counts were performed on peripheral blood samples from all subjects.

In vitro chemotaxis

Comparison of PMN chemotaxis in psoriatic smokers with psoriatic non-smokers and healthy non-psoriatic volunteers. Thirty-two smokers with chronic plaque psoriasis (20 female, 12 male; age range 19–71 years, mean 39) and 32 non-smokers (18 female, 14 male; age range 16–65 years, mean 35) with the same condition were studied. All 64 patients were free from other diseases, had no joint involvement, were not receiving any systemic medication and had used emollients only for the previous 2 weeks. All had between 25 and 50% body surface involvement, as assessed by taking the size of the patient's hand (fingers closed) to represent 1%.[9]

The smokers followed their usual pattern of cigarette smoking up to and during the study period and all smoked at least one cigarette during the hour prior to venesection.

These patients were sex and age matched (to within 5 years) with 32 control smokers and 32 control non-smokers.

Assessment of the effect of the numbers of cigarettes smoked on PMN chemotaxis. Twenty patients (12 female, eight male; age range 18 to 65 years, mean 34) with chronic plaque psoriasis who smoked more than 20 cigarettes per day were studied together with 20 sex and age matched (to within 5 years) psoriatic non-smokers and 20 sex and age matched (to within 5 years) psoriatics who smoked less than 20 cigarettes per day. All 60 patients were free of other diseases, had no joint involvement, were not receiving any systemic medication, had only used emollients for the previous 2 weeks and had between 25 and 50% of their body surface involved.

Twenty healthy adult non-smokers (12 female, eight male; age range 20–64 years, mean 35) were also studied together with 20 sex and aged matched (to within 5 years) healthy adults who smoked less than 20 cigarettes per day and 20 who smoked more than 20 cigarettes per day. A modification of the Boyden chamber technique[10] was used. The cell suspension was prepared by mixing 10 ml heparinized venous blood with 6 ml 6% dextran (molecular weight 110,000; Fisons Pharmaceuticals Ltd) and allowing the mixture to stand at room temperature for 45 min to sediment the red blood cells. The leukocyte rich supernatant was then drawn off, mixed with an equal volume of Hank's balanced salt solution (HBSS; Wellcome Reagents Ltd) and centrifuged at 400 *g* for 10 min. The cell pellet was resuspended in HBSS and adjusted to a final concentration of 2×10^6 leukocytes/ml.

E. coli endotoxin activated serum was used as the chemotactic agent in all cases. This was prepared by mixing 0·1 ml *E. coli* endotoxin (1·5 mg/ml, Sigma), 4·4 ml HBSS and 0·5 ml fresh autologous serum. This particular chemotaxin was chosen as it is the routine chemotaxin used in our laboratories for *in vitro* chemotaxis studies and is known to give consistent and reproducible results. The lower compartment of our modified Boyden chamber consisted of a 13 mm diameter Watmann AA disc in the well of a Sterilin migration plate. After soaking the disc with 0·8 ml of endotoxin activated serum, a 13 mm diameter filter of 3 μm pore size (Millipore UK Ltd) was applied directly to it. Non-stimulated controls were set up by adding 0·8 ml of HBSS to the Watmann AA discs.

The plastic upper compartment of our chamber was filled with 1·6 ml of the cell suspension and inverted directly onto the 3 μm filter covering the lower chamber. After incubation for 1 h at 37°C the filters were removed, fixed and stained and the distance from the surface of the filter to the leading front of cells (defined as the distance travelled by the farthest migrating five cells in the same plane), measured using a calibrated microscope. Measurements were made at five different points on the filter, and as each assay was performed in duplicate the results are expressed as a mean of 10 readings.

In vivo chemotaxis

A quantitative skin window chamber technique was used to study the effect of smoking on the chemotactic responsiveness of PMNs from eight psoriatic smokers (four males, four females; age range 20–58 years, mean 41·8 years), eight psoriatic non-smokers, eight healthy control smokers and eight healthy control non-smokers. The latter three groups were all sex and age matched (to within 5 years) with the group of psoriatic smokers.

All the psoriatic patients had between 25 and 50% of their total body surface involved. They had no joint involvement, were free from other diseases, were not taking any systemic medication and had only used emollients for the preceding 2 weeks.

The skin of the flexor aspect of the forearm was cleaned and, to aid abrasion, the stratum corneum was hydrated by contact with saline solution for 10 min. Four circular abrasions approximately 5 mm in diameter were made on uninvolved skin using a dental burr mounted in a hand held drill. The aim was to remove only superficial scale and epidermis until a glistening surface was produced. Bleeding did not occur in any subject. Each abrasion was then gently dabbed with dry, sterile cotton wool to removed fragments of keratin and covered with a cylindrical plastic chamber (volume 7 ml) that was cemented to the surrounding normal intact skin with a cyanoacrylate adhesive. Two of the chambers were filled with HBSS alone and two with 50% freshly collected untreated autologous serum in HBSS, 90% of the chemotactic activity of which is due to the complement fraction C_{5a}.[11]

The solutions were introduced through a small hole in the top of the chamber which could be securely stoppered when the chamber was full. Twenty four hours later the fluid in the chambers was aspirated (following a few seconds agitation of the fluid to release any adherent cells), and the cell content of the fluid measured using a haemocytometer. Each abrasion was then photographed and the individual areas measured by projection onto squared paper. The cell count per 10 mm^2 of abrasion per 24 h was then calculated. As all tests were performed in duplicate the results are expressed as the mean for each pair of chambers.

Statistical analysis

Student's *t*-test (2-tailed) was used for all analysis.

RESULTS

Total and differential white blood cell counts (including PMNs) from all subjects were within the normal range.

In vitro migration

No significant differences were found in either random PMN migration towards HBSS alone, or chemotaxis towards *E. coli* endotoxin activated serum, between psoriatic and control smokers, or between psoriatic and control non-smokers (Table 1). There were also no significant differences between control non-smokers and controls who smoked less than 20 cigarettes per day or more than 20 cigarettes per day, or between psoriatic non-smokers and psoriatics who smoked less than 20 cigarettes per day or more than 20 cigarettes per day (Table 2).

In vivo migration

No significant differences in random migration toward HBSS alone were found between control smokers, control non-smokers, psoriatic smokers and psoriatic non-smokers (Table 3).

There were also no significant differences between the chemotactic responses towards 50% autologous serum of PMNs from control smokers, control non-smokers and psoriatic non-smokers.

TABLE 1. *In vitro* random migration (HBSS alone) and chemotaxis towards *E. coli* endotoxin-activated serum of PMN from psoriatic and control smokers and non-smokers. Values are μm migrated, mean ± SEM

	Controls		Psoriatics	
	Non-smokers ($n=32$)	Smokers ($n=32$)	Non-smokers ($n=32$)	Smokers ($n=32$)
HBSS alone	39·38 ± 1·7	38·67 ± 1·93	41·50 ± 2·31	40·92 ± 2·17
Endotoxin activated serum	67·31 ± 2·37	66·23 ± 2·02	68·84 ± 2·5	66·13 ± 1·71

TABLE 2. *In vitro* random migration (HBSS alone) and chemotaxis towards *E. coli* endotoxin-activated serum, of PMN from psoriatic and control non-smokers and psoriatic and control smokers in relation to numbers of cigarettes smoked per day. Values are μm migrated, mean ± SEM

	Controls			Psoriatics		
	Non-smokers ($n=20$)	< 20 cigarettes per day ($n=20$)	> 20 cigarettes per day ($n=20$)	Non-smokers ($n=20$)	< 20 cigarettes per day ($n=20$)	> 20 cigarettes per day ($n=20$)
HBSS alone	38·69 ± 1·89	40·65 ± 2·26	38·45 ± 2·45	36·30 ± 2·8	42·65 ± 1·15	36·20 ± 2·11
Endotoxin activated serum	68·58 ± 2·18	68·20 ± 3·1	69·50 ± 2·73	64·62 ± 2·6	68·12 ± 1·98	66·41 ± 3·2

The chemotactic response towards 50% autologous serum of PMNs from psoriatic smokers was significantly greater than that occurring with PMNs from either psoriatic non-smokers or control smokers ($P < 0{\cdot}001$) (Table 3).

DISCUSSION

The recently demonstrated association between smoking and PPP, the probable relationship between PPP and psoriasis and the prominence of PMNs in both the histology and possibly the pathogenesis of both conditions stimulated the present investigations.

Our results showed that random migration of PMNs both *in vitro* and *in vivo* was unaffected by the presence of psoriasis or smoking, and the ability of PMNs from control subjects to respond, *in vitro* and *in vivo*, to the chemotaxins studied was unaffected by smoking. The ability of PMNs from non-smokers to respond, *in vitro* and *in vivo*, to the chemotaxins studied was unaffected by the presence of psoriasis. Also, *in vitro*, the response of PMNs from psoriatic patients to endotoxin activated serum was unaffected by smoking, and the response of PMNs from smokers was unaffected by the presence of psoriasis. However, *in vivo*, PMNs from psoriatic smokers demonstrated a significantly greater response towards 50% autologous serum than PMNs from either control smokers or psoriatic non-smokers.

The *in vivo* 'hyperresponsiveness' of PMNs from psoriatic smokers may not necessarily be due to smoking. Although we tried to match our groups for as many variables as possible (i.e. sex, age and severity of psoriasis), and alcohol consumption was prohibited, there are other factors, such as personality, stress and dietary habits that may effect one group more than another.

The finding that PMNs from psoriatic smokers were only demonstrably hyperresponsive *in vivo* and not *in vitro* may merely reflect a difference in the sensitivity of the techniques employed or a selectivity of response to different chemotaxins. It may be that psoriatic PMNs have an affinity for C_{5a} that is selectively enhanced by smoking. Alternatively, it could indicate that the effect was dependent on interactions with other cutaneous constituents rather than simply being due to a defect in the PMNs themselves. The *in vitro* technique used PMNs from peripheral

TABLE 3. *In vivo* random migration (HBSS alone) and chemotaxis towards 50% autologous serum, of PMN in control and psoriatic smokers and non-smokers

	No of cells × 10^6/10 mm^2/24 h (mean ± SEM)	
	HBSS alone	50% autologous serum
Controls		
Non-smokers ($n=8$)	2·80 ± 0·50	19·08 ± 3·52
Smokers ($n=8$)	2·17 ± 0·32	22·10 ± 6·22
Psoriatics		
Non-smokers ($n=8$)	2·46 ± 0·41	16·86 ± 3·44
Smokers ($n=8$)	3·33 ± 0·57	41·61 ± 3·19*

* Significantly greater than in psoriatic non-smokers and control smokers ($P < 0{\cdot}001$).

venous blood. These may not necessarily be representative of those present within psoriatic plaques, which may be a different or specially selected subpopulation with different properties.[12,13]

Our studies used a single concentration of each chemoattractant, and concentrations were selected to enable comparison with previous studies performed by us and with those reported in the literature. Since chemotaxis dose response curves are bell-shaped, the peak response for PMNs in the groups of subjects studied could differ. This, however, would still indicate that a functional difference exists in PMNs from psoriatic smokers.

An abnormal response of PMN chemotaxis in psoriatic smokers could be due to an inherent defect of the PMN itself, the presence of 'local' factors capable of increasing the sensitivity of the psoriatic PMN or a combination of the two. It would appear unlikely that there is an inherent defect in the PMNs of psoriatic smokers and not in psoriatic non-smokers since this implies a genetic basis for both PMN chemotactic hypersensitivity and becoming a smoker. A more probable explanation is that a factor present in the skin or blood of smokers interacts with the psoriatic PMNs, which in turn are inherently different to 'normal' PMNs, and they are thus rendered chemotactically hyperresponsive. Such PMNs may then respond inappropriately to normal cutaneous constituents or demonstrate an exaggerated response to chemoattactants that have been shown to be present within psoriatic lesions.[14,15] Tagami and Ofuji[16] postulated that one of the chemoattractant agents demonstrable within psoriatic scale was derived from complement, possibly being a C_5 cleavage product. This may be particularly relevant to our *in vivo* findings since 90% of the chemotactic activity of autologous serum is due to the complement fraction C_{5a}.[11]

Having accumulated within the skin, PMNs have the potential to produce both the inflammatory[17] and the hyperplastic components[18] of the typical psoriatic lesion.

Reports that smoking stimulates neutrophil superoxide release,[19] alters phagocytic oxidative metabolism[20,21] and increases spontaneous PMN nitroblue reduction[22] suggest that apart from being associated with increased chemoattraction of PMN in psoriasis, smoking may also increase the release of oxygen metabolites and enzymes capable of causing a marked inflammatory reaction.

The *in vivo* finding that PMNs from psoriatic smokers are significantly more responsive to a chemotaxin than PMNs from psoriatic non-smokers or control smokers may provide an explanation for the association of palmoplantar pustulosis with smoking and should stimulate further work into the possible relationship between smoking, PMN function and psoriasis vulgaris. Future *in vivo* studies of PMN function should take smoking into account when psoriatic and control subjects are being compared.

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Distribution of EGF receptor expressing and DNA replicating epidermal cells in psoriasis vulgaris and Bowen's disease

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SUMMARY

We have examined the localization of DNA replicating cells and EGF receptor-expressing cells in the epidermis of psoriasis vulgaris, a benign hyperproliferative skin disease, and Bowen's disease, a pre-malignant hyperproliferative skin disease, and normal skin. DNA replicating cells were detected by anti-BrdU monoclonal antibody after incubating tissue sections with BrdU, and EGF receptors were detected by the anti-EGF receptor monoclonal antibody B4G7. In normal skin, DNA replicating cells were localized exclusively in the basal and suprabasal layers. EGF receptor expression was observed most strongly in the basal and parabasal layers, but diminished gradually towards the upper squamous layer. In psoriatic skin, DNA replicating cells were also localized in the basal and parabasal layers, but the number of these mitotic cells was about 10 times higher than in normal skin. In this case, more EGF receptors were detected in all viable layers of the epidermis. Apparently normal skin adjacent to psoriasis lesions showed persistent expression of EGF receptors in the upper squamous layer without an increased number of DNA replicating cells in the basal and parabasal layers. In Bowen's disease, DNA replicating cells and EGF receptor expressing cells were distributed in all layers of the epidermis. These findings indicate that the increased production of EGF receptors may be, in part, responsible for the hyperproliferative state of the epidermis and that cells in the upper squamous layer of psoriasis may have lost a mechanism by which EGF receptor expression is diminished thus allowing differentiation. This altered process of EGF receptor production may be involved in the onset of psoriasis vulgaris.

Epidermal growth factor (EGF) is a potent mitogen for a variety of mammalian epithelial tissues and cells. There is evidence to indicate that the initial interaction of EGF with target cells occurs

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on specific membrane receptors, i.e. EGF receptors.[1] In normal human epidermis, EGF receptors are localized primarily on the mitotically active basal keratinocytes and diminish in number as the cells differentiate. EGF receptors are usually detected by autoradiography of [^{125}I]-EGF-labelled specimens or immunohistochemistry using anti-EGF receptor antibody.[2-5] Correlations between EGF receptors and cellular proliferation made by these methods are imperfect because EGF receptors are present on mitotically inactive, but metabolically active cells such as sweat duct epithelium, arrector pili muscles, and myoepithelial cells of normal human skin.[3] By contrast, in the present study, localization of the proliferative DNA synthesizing cells was detected by anti-BrdU monoclonal antibody after incubating tissues with the thymidine analogue BrdU. The distribution of EGF receptors was detected by anti-EGF receptor monoclonal antibody B4G7.[6] These methods allowed us to examine the relationship between EGF receptors and cellular proliferation in benign and pre-malignant hyperproliferative conditions of the epidermis. We chose psoriasis vulgaris as the benign hyperproliferative condition and Bowen's disease as the pre-malignant hyperproliferative condition.

METHODS

Materials

The B4G7 monoclonal antibody specific to the human EGF receptor has been described previously.[6] The Vectastain ABC kit and biotinylated horse anti-mouse IgG were obtained from Vector (Burlingame, CA, U.S.A), OCT compound from Miles Laboratory (Naperville, IL, U.S.A.) and BrdU and anti-BrdU monoclonal antibody from Ikeda Rika (Osaka, Japan) and Becton Dickinson (Mountainview, CA, U.S.A.), respectively. Normal mouse IgG was obtained from Sigma (St. Louis, MO, U.S.A.). All other reagents used were of analytical grade.

Specimens

Normal skin specimens were obtained from patients undergoing elective plastic surgery at Keio University Hospital. Skin biopsies were obtained using 1% xylocain anaesthesia from 14 patients with psoriasis vulgaris and four patients with Bowen's disease, from involved and uninvolved skin, before starting any therapy.

BrdU staining

DNA replicating cells were detected by the ABC (Avidin-Biotin Complex) method using anti-BrdU monoclonal antibody after incubating tissues with BrdU. Tissue samples were placed in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) at 4°C and cut into 1 mm slices. BrdU was added to a final concentration of 400 μM and the samples were incubated at 37°C under pressure of 3 kgw/cm^2 for 2 h, fixed in 70% ethanol, embedded in paraffin, and cut into 2 μm sections. The sections were then de-paraffinized, immersed in 2 M HCl for 30 min to denature DNA, and neutralized for 10 min in 0·1 M $Na_2B_4O_7$. The sections were then covered with 10% normal horse serum at room temperature for 30 min, and incubated with anti-BrdU monoclonal antibody (diluted 1:100 with 10% normal horse serum) at 4°C overnight. The subsequent steps were carried out according to the usual ABC staining method. The sections were stained with diaminobenzidine (0·5 mg/ml) in 0·01% H_2O_2 and counterstained with methyl green. Normal mouse IgG was used as a negative control.

Labelling indices were obtained as follows:[7] 2000 nuclei of epidermal cells (i.e. cells of both stratum basale and stratum spinosum) were counted and classified as labelled or unlabelled. The labelling indices were then calculated from the equation:

$$\text{Epidermal cell labelling index} = \frac{\text{Number of labelled epidermal cell nuclei}}{\text{Number of epidermal cell nuclei counted}} \times 100$$

EGF receptor staining

EGF receptors were detected by the ABC method using B4G7 mouse anti-human EGF receptor IgG monoclonal antibody as previously reported.[8] For this purpose, tissues were embedded in OCT compound, snap-frozen and 6 μm cryostat section were cut. The sections were then fixed in ice-cold acetone, covered with 10% normal horse serum at room temperature for 30 min, and then incubated with B4G7 antibody (1 μg/ml in TBS) for 30 min at room temperature. The subsequent steps were the same as for BrdU staining.

RESULTS

Distribution of DNA replicating cells

Cells that had incorporated BrdU into DNA were detected by immunohistochemical staining using anti-BrdU monoclonal antibody. Figures 1A, C, E and G show the distribution of nuclei which had incorporated BrdU in different skin conditions. The DNA replicating cells were localized exclusively in the basal and suprabasal cell layers in both normal and psoriatic skin (Fig. 1A and C). The number of labelled nuclei in lesional psoriatic skin, however, was 10 times greater than in normal skin (see also Table 1). In lesions of Bowen's disease, DNA replicating cells were found in all epidermal layers (Fig. 1E).

Distribution of EGF receptors

EGF receptors were visualized by the anti-EGF receptor monoclonal antibody B4G7. Figures 1B, D, F and H show the localization of immunoreactive EGF receptors in the different skin

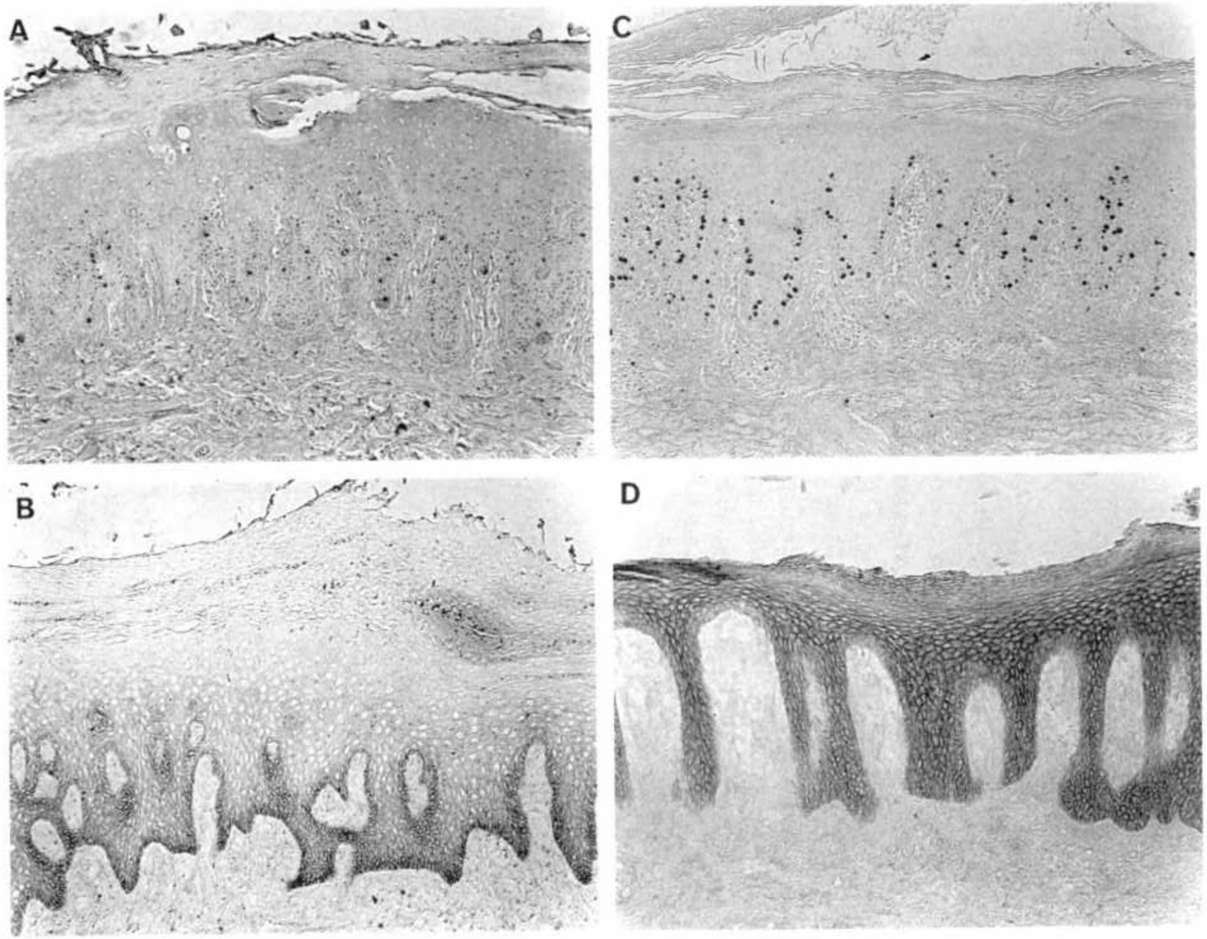
TABLE 1. Immunohistochemical findings in patients with psoriasis vulgaris and Bowen's disease

		Psoriasis vulgaris (benign)		
	Normal sole skin	Uninvolved*	Involved	Bowen's disease (pre-malignant)
Number of cases	8	5	14	4
Labelling index† (mean ± SD)	2·4 ± 0·5	5·8 ± 1·0‡	21·3 ± 2·6‡	27·8 ± 4·2
Localization of DNA synthesizing cells	basal and parabasal layers	basal and parabasal layers	basal and parabasal layers	all layers
Distribution of EGF-receptor positive cells	basal and parabasal layers	all layers	all layers	all layers

* Clinically normal skin adjacent to active lesions.

† 2000 nuclei were counted.

‡ Average of seven cases of psoriasis vulgaris.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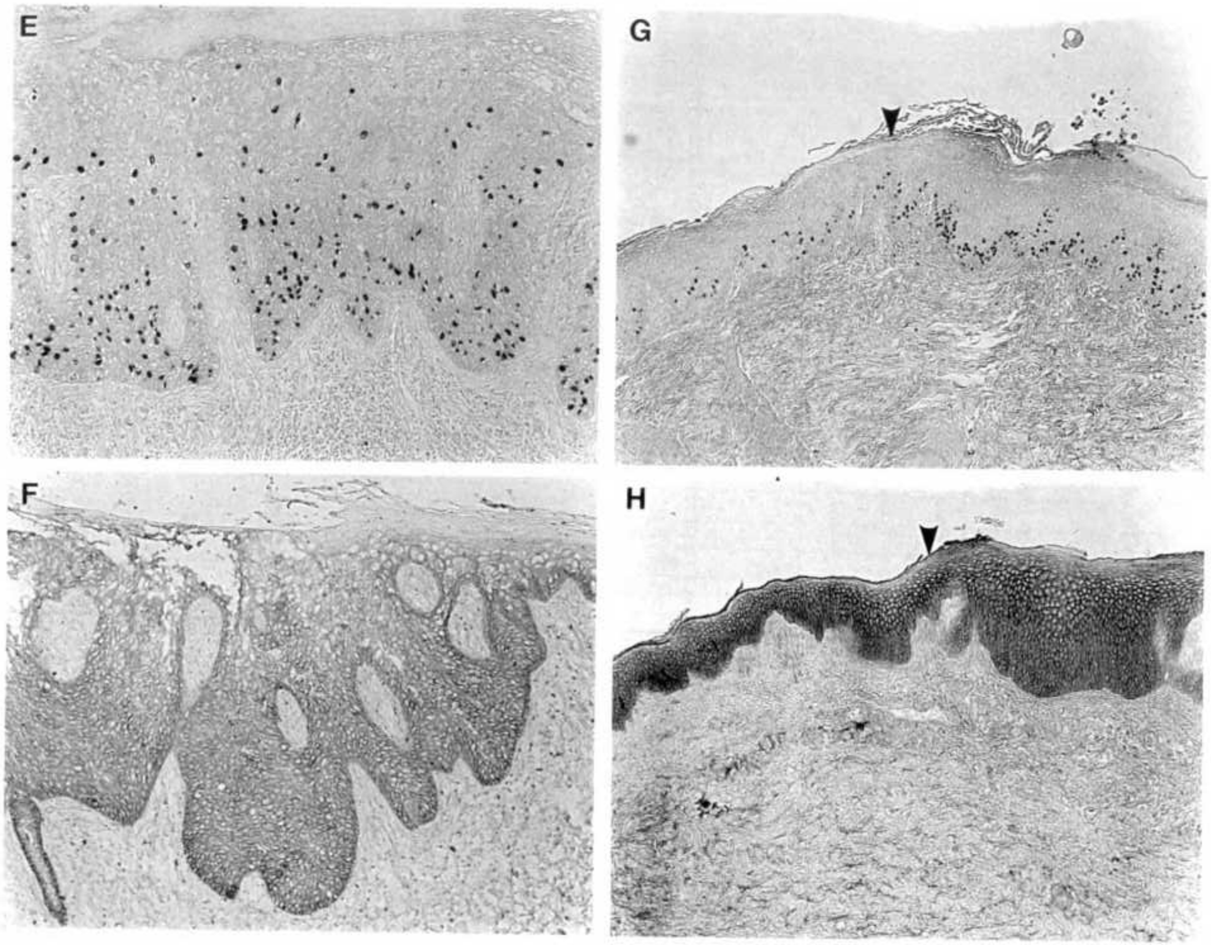

FIGURE 1. Immunohistochemical staining of BrdU containing and EGF receptor-positive cells. (A) (C) (E) and (G) localization of DNA synthesizing cells; (B) (D) (F) and (H) distribution of EGF receptors. (A) and (B): normal sole skin; C and D: lesional skin of psoriasis vulgaris; (E) and (F): lesional skin of Bowen's disease; (G) and (H): clinically normal skin adjacent to lesion of psoriasis vulgaris (arrows mark junction of normal and lesional epidermis). (A–F: Original × 100, G and H: Original × 65).

TABLE 2. Clinical features of 14 patients with psoriasis vulgaris

Patient no.	Age (years)	Sex	Clinical type	Distribution of skin lesions	Site of biopsy	Duration of disease
1	36	M	Nummular	Extremity	Dorsum of hand	2 years
2	22	M	Nummular	Extremity	Posterior cubital	2 years
3	48	M	Nummular	Extremity, Trunk	Gluteal	2 years
4	36	F	Nummular	Extremity	Posterior cubital	3 years
5	43	F	Nummular	Head, Neck	Nuchal	1 year
6	63	M	Nummular	Extremity	Lateral brachial	6 months
7	29	M	Nummular	Extremity, Trunk	Scapular	6 years
8	64	M	Nummular	Head, Extremity, Trunk	Scapular	10 years
9	71	F	Nummular	Lower extremity	Anterior crural	2 years
10	47	M	Nummular	Upper extremity	Posterior cubital	5 years
11	52	M	Nummular	Extremity	Posterior cubital	3 years
12	69	M	Guttate	Extremity, Penis	Posterior cubital	6 months
13	83	M	Nummular	Trunk, Head, Extremity	Lumbar	10 years
14	19	M	Nummular	Extremity, Head	Posterior cubital	7 years

conditions. In normal sole skin, EGF receptor expression was mostly in the basal and suprabasal layers, but gradually diminished towards the upper squamous layer (Fig. 1B). In lesional psoriatic skin, more EGF receptors were detected not only in the basal and parabasal layers, but also in the upper squamous layers (Fig. 1D). In Bowen's disease, tumour cells in all layers of the epidermis were stained (Fig. 1F). The EGF receptor-positive cells in general showed staining of the membrane, and the cytoplasm was only weakly stained.

Figure 1G shows that there is a clear border between involved skin and uninvolved skin in psoriasis vulgaris, judging from the number of DNA replicating cells and pathological findings such as acanthosis and elongation of rete ridges. Uninvolved psoriatic skin exhibited a reduced number of proliferating cells as compared with involved skin. However, EGF receptors were distributed widely from the basal layer toward the upper squamous layer. This suggests that EGF receptors may begin to persist throughout the epidermis before it becomes clinically psoriatic.

Table 2 summarizes the clinical features of the 14 patients with psoriasis vulgaris. The distributions of proliferating cells and EGF receptor expressing cells were constant in these patients regardless of age, sex, clinical type, distribution of skin lesions or duration of illness.

DISCUSSION

The localization of proliferative cells in normal skin, psoriatic skin and a variety of skin tumours has been reported.[7,9–12] Autoradiography with [^{3}H]-thymidine has been used *in vivo* and *in vitro* to identify DNA synthesizing cells in all of the above cases. In the present study, an immunohistochemical staining procedure with BrdU was used for the same purpose because of its simplicity and rapidity.

In normal skin, DNA replicating cells were localized exclusively in the basal and suprabasal layers. EGF receptors were expressed most strongly on these basal and suprabasal layers, but diminished in number towards the upper squamous layer. In lesional psoriatic skin, DNA replicating cells were also localized in the basal and parabasal layers, but the number of these cells was approximately 10 times higher than in normal skin. EGF receptors were detected throughout all viable layers of the epidermis. Nanney *et al.*[13] reported that immunoreactive EGF receptors were present throughout the psoriatic epidermis including the stratum corneum. In our study, the stratum corneum was not stained with anti-EGF receptor antibody and the staining intensity of the cytoplasm was much weaker than that of the cell membrane. This apparent discrepancy may be due to the use of different antibodies. Our B4G7 antibody interacts with a protein portion of the EGF receptor whereas other antibodies currently in use interact with oligosaccharide moieties. Also, using the B4G7 antibody method, we have examined tissue of lamellar ichthyosis, which is known to have a marked increase in epidermal cell proliferation,[14,15] and have observed similar results to those seen in psoriatic skin (data not shown). Thus, the use of B4G7 antibody may be of considerable advantage in examining the distribution of EGF receptors to indicate onset of a variety of skin disorders.

In clinically normal skin adjacent to the lesions of psoriasis vulgaris, EGF receptor-positive cells were found even in the upper squamous layers, although no increase of cell turnover rate was seen, judging from the number of proliferating cells and lack of either acanthosis or elongation of rete ridges. This finding indicates that persistence of EGF receptors is not a result of increased epidermal cell turnover, but is likely to be responsible for the hyperproliferative state of psoriasis.

In the epidermis of Bowen's disease, which represents the pre-malignant hyperproliferative state, DNA replicating cells were diffusely distributed and the distribution of proliferating cells was consistent with that of EGF receptor-positive cells at the tissue level. However, the upper squamous layers of lesions of psoriasis vulgaris showed EGF receptor-positive cells which were not proliferative. This indicates that squamous cells in the upper layer of psoriasis cannot proliferate even though they have EGF receptors. Therefore, it is possible that these EGF receptors neither transmit the mitogenic signal nor are involved in the mechanism which makes cells enter into differentiation. It is also possible that an altered process of EGF receptor production may be involved in the onset of psoriasis vulgaris. Further studies will be necessary to clarify these mechanisms.

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Acne vulgaris and malignancy

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SUMMARY

Seventy patients with lymphomas and leukaemias were interviewed. Details of past acne vulgaris were recorded and a clinical examination performed. A history of acne was less common in the patients than in matched controls. In addition, fewer patients had received treatment prescribed by a medical practitioner for their acne than had the controls. Examination revealed fewer patients with acne. These results suggest a reduced incidence of acne or its more severe forms in patients who have developed lymphomas and leukemias, and provide some support for the hypothesis that increased exposure or response to *P. acnes*, as appears to occur with acne, could confer protection against malignant disease.

Administration of *Corynebacterium parvum* to animals results in stimulation of the reticulo-endothelial system.[1] In addition, anti-tumour activity has been demonstrated against a variety of neoplasms in animals, in particular leukaemias, melanoma, and ovarian and breast carcinomas.[1] It is now accepted that the gram positive bacteria *C. parvum* and *Propionibacterium acnes* are one and the same organism.[2] It is possible that exposure to greater numbers of, or an enhanced response to, these organisms may confer benefits in man, such as a reduced susceptibility to malignant diseases.

P. acnes is strongly implicated as a factor in the pathogenesis of acne. Although there is controversy as to whether or not surface levels of this organism are increased,[3,4] it has been shown that greater proportions of inflamed and non-inflamed acne lesions contain *P. acnes* than do normal pilosebaceous follicles.[5] It has also been observed that individuals with acne respond excessively to some *P. acnes* antigens.[6]

If individuals with acne, by virtue of increased exposure or increased responsiveness to *P. acnes*, were to have enhanced immune function, it might be expected that people who develop malignant disease in adolescence or early adult life would have had less acne or less severe acne than the normal population. We, therefore, investigated the frequency and severity of acne in patients with lymphomas and leukaemias, and compared this data with data from the normal population.

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METHODS

Seventy patients with malignant diseases were interviewed. Their diagnoses were: Hodgkin's disease (44); non-Hodgkin's lymphoma (7); acute myeloid leukaemia (9); acute lymphoblastic leukaemia (9); chronic granulocytic leukaemia (1). Most patients were visited at home or in oncology clinics to avoid adding to already frequent hospital visits. The observer was, therefore, by necessity aware of the patient or control status of each subject. Control subjects were individuals visiting hospital in-patients. These subjects were approached by ward staff who were unaware of the reasons for the study in order to minimize selection bias. None of the subjects were aware of the reasons for the study. All patients and controls were from the Leeds–Bradford area.

Patients were asked to complete a questionnaire. Details of skin disorders before diagnosis of malignant disease were obtained, with particular reference to acne (whatever the severity) and its treatment (whether this was self-treatment or prescribed by a medical practitioner). The details of any anticancer therapy (including corticosteroids, cytotoxic chemotherapy and radiotherapy) were also noted. Control subjects completed a similar questionnaire.

An examination of the face, back and chest was performed by a single observer. The presence of acne was noted and a severity grade assigned for each site on a scale of 0–10.[7] The sum of the grades for these three areas was calculated to give a total acne grade for each subject. The presence of acne scarring was also noted. Subjects with a total acne grade of $<0{\cdot}375$ without evidence of scarring were considered to have 'physiological acne'. Subjects with a total acne grade of $>0{\cdot}375$ (i.e. acne severity for which topical or systemic therapy would normally be prescribed) or with evidence of scarring, were considered to have 'clinical acne'.

Patients and controls were matched for sex and age (within 5-year age intervals from 15–39 years). Questionnaire data from patients was compared with controls matched for age at diagnosis of malignant disease. Data from acne grading of patients was compared with controls matched for age at the time of the study. Acneiform eruptions can be produced by administration of corticosteroids both topically and systemically, and can persist some months after their use.[8] Acne has also been reported in a significant proportion of subjects receiving cyclosporin A, most frequently between 2 and 6 months after therapy.[9] In addition, acneiform eruptions have been described up to 6 months after radiotherapy at the site of treatment, even when skin surface sparing techniques are employed.[10] We, therefore, excluded from analysis of acne grading, patients who had received these modes of therapy within 6 months of the study.

Relative risk (RR) and statistical significance were determined by the Mantel-Haenzel procedure using the programmes of Rothman and Boice.[11] Cases and controls were compared for age using Student's t-tests.

RESULTS

Questionnaire

The incidence of the most frequently reported skin disorders in the 70 patients and controls are given in Table 1. The two groups each consisted of 35 male and 35 female subjects. The mean ages (± SD) of patients at diagnosis and of the controls were 23·4 (± 4·9) and 23·2 (± 4·7) years, respectively. There was no significant difference between cases and controls for mean age in each 5-year age category. The mean delay (± SD) between diagnosis and inclusion of patients in the study was 3·8 (± 3·51) years.

The proportion of patients who gave a history of acne prior to diagnosis of their malignant

TABLE 1. History of skin disorders in patients and controls

Skin disorder	Leukaemia/lymphoma group ($n=70$) No. (% of total)	Control group ($n=70$) No. (% of total)	Relative risk	95% confidence limits	Two-tail *P*-value
Acne	40 (57·1%)	50 (71·4%)	0·52	0·26–1·07	0·08
Eczema	9 (12·9%)	9 (12·9%)	1·00	0·37–2·69	0·80
Psoriasis	3 (4·3%)	4 (5·7%)	0·75	0·17–3·33	0·70

disease was less than that of the controls (RR = 0·52), but the difference was not significant. The proportions of patients and controls with histories of eczema and psoriasis were not significantly different.

The details of acne treatment prior to diagnosis of malignant disease are given in Table 2. Similar proportions of patients and controls had either obtained no treatment for their acne or had treated themselves with over-the-counter preparations. However, fewer patients than controls had received prescribed treatment (either oral antibiotics or topical therapy alone) from a medical practitioner for their acne (RR = 0·15, two-tail $P=0·01$). A significant overall trend was observed using the Mantel-Haenzel extension test for trend (two-tail $P=0·02$).

Physical examination

The data for acne severity are shown in Table 3. Twenty-six patients who had received corticosteroids, cyclosporin A or radiotherapy within 6 months of the study were excluded. The remaining 44 patients consisted of 21 males and 23 females, mean age (± SD) 28·3 (± 5·08) years. As these numbers were relatively small, controls were matched in a ratio of 1 : 2. The mean

TABLE 2. Use of treatments for acne in patients and controls: frequency and relative risks

Acne treatment	Leukaemia/lymphoma group ($n=70$) No. (% of total)	Control group ($n=70$) No. (% of total)	Relative risk	95% confidence limits	Two-tail *P*-value
No acne	30 (42·9%)	20 (28·6%)	1·00	—	—
No treatment	18 (25·7%)	18 (25·7%)	0·67	0·28–1·59	0·48
Self treatment	19 (27·1%)	19 (27·1%)	0·67	0·28–1·57	0·48
Prescribed treatment	3 (4·3%)	13 (18·6%)	0·15	0·04–0·56	0·01

TABLE 3. Acne severity in patients and controls

Acne severity	Leukaemia/lymphoma group ($n=44$) No. (% of total)	Control group ($n=88$) No. (% of total)	Relative risk	95% confidence limits	Two-tail *P*-value
No acne	34 (77·3%)	50 (56·8%)	1·00	—	—
Physiological acne	8 (18·2%)	32 (36·4%)	0·37	0·15–0·88	0·04
Clinical acne	2 (4·5%)	6 (6·8%)	0·49	0·10–2·53	0·64

age (± SD) of the control group was 28·1 (± 5·45) years. There was no significant difference in mean age between patients and controls for each 5-year age category.

Fewer patients (in most cases after anticancer therapy) had physiological acne than controls (RR = 0·37, two-tail $P = 0{\cdot}04$). In addition, fewer patients had clinical acne than controls (RR = 0·49), but this was not statistically significant.

DISCUSSION

There is firm evidence that *C. parvum* (*P. acnes*) promotes anticancer activity in animals. This has been demonstrated with exposure to *C. parvum* both before and after exposure to the tumour. The mode of action is unclear. There appears to be a non-specific action possibly mediated by macrophage activation. In addition, enhancement of specific cell mediated responses to tumour antigens may occur. It appears that these effects increase with increasing specific immune response to *C. parvum* and possibly with increasing doses of this organism.[1] Experience with *C. parvum* (*P. acnes*) vaccine as a therapeutic agent in man is limited. However, clinical benefits have been reported in some patients with advanced malignancies.[12]

Acne is a common condition affecting the majority of Caucasians to some degree during early life. Shuster[13] commented on the high incidence of acne and put forward an hypothesis to explain its persistence in the population. He proposed that enhancement of immune function by *P. acnes* might occur in patients with acne, and that this biological purpose of acne could favour its persistence by natural selection. Beral *et al.*[14] analysed cutaneous risk factors for malignant melanoma and found fewer patients than expected with a history of acne in the melanoma group. Paslin[15] suggested that *P. acnes*-induced enhancement of immune function might protect against development of melanoma, and put this forward as an explanation of Beral's results. In a subsequent study by Elwood *et al.*[16] no association between acne and melanoma was found.

In our study, a history of acne (before diagnosis of malignant disease) was reported by slightly smaller numbers of patients than controls, although this difference was not significant. In addition, significantly fewer patients had received medication for acne from a medical practitioner. This may indicate that severe acne was less common amongst patients. There is indeed evidence to suggest that subjects with acne who consult a doctor have a high incidence of severe acne grades.[17] Examination (in most cases after treatment for malignant disease) revealed significantly less physiological acne amongst patients than controls. Although 'clinical' acne was less common in patients than controls, the difference was not significant.

Reliance on histories to obtain details of a common and often minor disability such as acne introduces a potentially significant source of error. The time delay between diagnosis and the study may have made recall more difficult for patients than the controls and this could have affected our results. Patients own diagnoses of acne may have been incorrect and their assessments of requirement for therapy are clearly subjective. However, a more reliable diagnosis of acne would be expected in those who had consulted a medical practitioner and subsequently received specific therapy for acne. Factors which might influence the likelihood of a subject presenting to a medical practitioner for treatment of acne, such as socio-economic status, were not controlled for and could have led to error in our results.

Our procedure for obtaining control subjects may also have introduced bias. However, the incidence of acne in our control group (43·2%) is in keeping with the incidence reported in another study which examined subjects from the same geographic area.[18] In this study, facial acne of all grades ranged from 90% at 18 years to 30% at 35 years and did not include truncal acne which may occur in the absence of facial acne. The incidence of prescribed treatment for acne in our controls was 26% of those with a history of acne. This figure is consistent with the data reported by Elwood *et al.* for their control group (18·5% of subjects with a history of acne had received prescribed creams and 9·3% had received oral medication)[16] and is similar to our own experience i.e. 20% of subjects with acne having received prescribed therapy (W.J.Cunliffe, unpublished observations).

Corticosteroids, cyclosporin A and radiotherapy may cause acneiform eruptions in some patients during or several months after treatment. The possibility that these agents could improve acne in other patients cannot be excluded. Whether or not other cytotoxic agents could have had an effect on acne status in our patients cannot be determined. The observer was not blind to the case or control status of subjects and this may have resulted in bias in assessment of acne grades.

Although definite conclusions cannot be drawn from our results, they do lend support to the suggestion that the acne process may confer some protection against development of malignant disease. The acne process could also confer protection against some infections, as suggested by *P. acnes*-enhanced resistance to some bacterial infections in animals.[19] If these proposed benefits of acne could be demonstrated by further study, this would have implications for our approach to the management of acne, particularly in its less severe forms.

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Binding of nickel to human epidermis *in vitro*

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SUMMARY

Human epidermis was homogenized, dried and incubated with nickel chloride solutions in the concentration range 0·008–3·4 mM/50 mg epidermis. Nickel uptake was found to occur according to the Freundlich adsorption isotherm, giving a slope of 0·55, indicating that nickel is associatively bound to constituents of the epidermis.

The binding characteristics of cobalt were very similar to those of nickel, but the two metals were not found to compete significantly for binding sites in the epidermis. The metal-chelating agents, ethylenediamine tetra-acetic acid disodium, (Na-EDTA) L-histidine and D-penicillamine were tested for ability to remove nickel from its binding sites. Na-EDTA was found to be the most efficient, removing 70–90% of the nickel bound to the tissue. The possible clinical significance of these *in vitro* observations are discussed in relation to percutaneous absorption and allergic contact dermatitis.

The epidermis together with the stratum corneum is the primary and most effective barrier to percutaneous penetration of chemicals. Moreover, some chemicals interact with the epidermal tissue in the process of percutaneous penetration. Epidermal binding characteristics of several organic compounds have been reported.[1] In recent studies we have shown that nickel penetrates the skin very slowly, with a lag-time of about 50 h.[2,3] Furthermore, a considerable amount of nickel was found in isolated epidermis when a steady-state had been established. Retention of nickel by epidermal tissue indicates that this layer possesses a specific affinity for nickel, which it binds reversibly. The binding of nickel ions (Ni^{2+}) to human dermis has been studied *in vitro* by Spruit *et al*,[4] who found a reversible binding of nickel to dermis. Samitz and Katz[5] in a later study found considerable nickel uptake by powdered epidermis and proposed that carboxyl groups are involved in the binding of nickel to a greater extent than are amino groups.

The objective of the present work was to study the epidermal binding of nickel. The study involved nickel uptake by powdered human epidermis and study of the possible ability of three chelating agents (Na-EDTA, D-penicillamine and L-histamine) to remove nickel bound to human epidermis.

Cobalt allergy is frequently found in nickel sensitive patients.[6–8] The high prevalence of co-

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sensitivity to cobalt may indicate that the source of sensitization is related.[9] Therefore, cobalt was included in the binding study in order to study a possible relationship between the binding of nickel and of cobalt.

METHODS

Chemicals

Nickel chloride hexahydrate, cobalt chloride hexahydrate, L-histidine monohydrochloride, D-penicillamine, ethylenediamine tetra-acetic acid disodium (disodium-EDTA) and tris(hydroxymethyl)-amino methane were all of analytical grade; sodium chloride and hydrochloric acid were suprapur grade quality. (All chemicals were obtained from Merck, Darmstadt, F.R.G.).

The water used was purified by a Milli-Q deionization system (Millipore Corporation, Bedford, MA, U.S.A.). Before use all containers and utensils were soaked in 4M nitric acid for several days and then rinsed with copious amounts of deionized water.

Incubation media

The incubation medium consisted of Tris-HCl-buffer (0·1 M) pH = 7·4 containing 0·15 M NaCl with varying amounts of nickel chloride or cobalt chloride or both. To some of the nickel solutions, chelating agents were added, making the molar ratio of chelate to nickel equal to 5. Three chelating agents which form water soluble chelates with nickel were tested, L-histidine D-penicillamine and Na-EDTA. The nickel concentrations employed were in the range 0·5 μg/ml (8·5 μM), to 200 μg/ml (3·4mM).

Skin preparations

Human skin from individuals undergoing surgery was used. Epidermis was separated from dermis by incubation of the skin in a dessicator for 5 min at 60°C and 100% relative humidity and the surface washed with saline. The isolated epidermis was placed in a teflon flask containing a grinding ball made of teflon with a steel core, and cooled in liquid nitrogen. The frozen material was homogenized using a laboratory ball mill (Mikro-Dis-Membrator II, B. Braun Melsungen A.G.). The powdered epidermis was collected, freeze dried and kept in a dessicator.

Binding studies

Portions of 50 mg dry powdered epidermis were weighed into separate test tubes and 5 ml of incubation medium was added to each tube. After incubation in a rotary shaker at 37 ± 1°C for 48 h, the tubes were centrifuged. An equilibrium time of 48 h was used to ensure the attainment of a constant nickel concentration in the incubation medium. A blank without the powdered epidermis served as control at each nickel concentration. The supernatants were taken off for nickel analysis and the amount of nickel bound to the epidermis was calculated by subtracting the quantity of nickel in the incubation medium at equilibrium from the amount of nickel in the controls. Dividing this amount by the weight of powdered epidermis gave the concentrations (w/w) of nickel bound to the powdered epidermis. Each experiment was performed in duplicate.

Nickel analysis

Nickel was quantified by adsorption differential pulse voltammetry (ADPV). The technique has been described by Pihlar *et al.*[10] It involves adsorption of the chelate nickel bis-dimethylglyoxymate onto a hanging mercury drop electrode in an ammonia buffer at pH 9·2, followed by a differential pulse scan in the cathodic direction. The limit of detection is 1 ng/l. Cobalt was quantified using the same technique. The instrumentation, conditions, and reagents used have been described previously.[11] All analytical manipulations took place inside a laminar airflow clean bench. The media containing the chelates were wet ashed as previously described[3] prior to nickel analysis.

RESULTS

Binding of nickel

The results of the nickel binding experiments are listed in Table 1. It appears that there was not simple partition of nickel between the two phases, the epidermis and the aqueous medium, since the ratio of nickel in epidermis to that in the incubation medium was highly dependent on the initial concentration. Simple partition would give an almost linear relationship between the concentrations of nickel in the epidermis and the aqueous phase. The binding can be described mathematically by the Freundlich adsorption equation:

TABLE 1. Binding of nickel to powdered human epidermis

Nickel concentration in incubation medium				
Initial (μg/ml)	Equilibrium (μg/ml)	% change	Nickel uptake by epidermis (μg Ni/g)	Nickel concentration ratio at equilibrium epidermis: incubation medium
181·5	136·9	−24·6	4359	31·8
91·9	62·0	−32·5	2839	45·8
41·5	28·7	−30·8	1249	43·5
26·4	12·4	−53·0	1335	108
8·99	4·24	−52·8	467	110
4·47	1·96	−56·1	246	126
0·960	0·114	−88·1	83	728
0·459	0·031	−93·2	42	1355

$$\log m = \log K + n \log c$$

where m is the amount of metal bound per gram of epidermis, K and n are system constants and c is the concentration of unbound metal in the surrounding solution at equilibrium. In Figure 1 the results have been plotted according to the Freundlich adsorption equation; the best straight line through the experimental data was calculated by the least-squares method. Evidently, there is a linear relationship between log m and log c. The values of n and K were determined from the slope and intercept of the regression line, respectively. The n value was 0·55, and K was 251 evaluated in μg/g. The correlation coefficient was 0·989. Since the n value was significantly less than one, the data indicate that nickel was associatively bound to one or more constituents of the epidermis.[1]

Binding of cobalt

Figures 2 and 3 show the binding of nickel and cobalt plotted according to the Freundlich

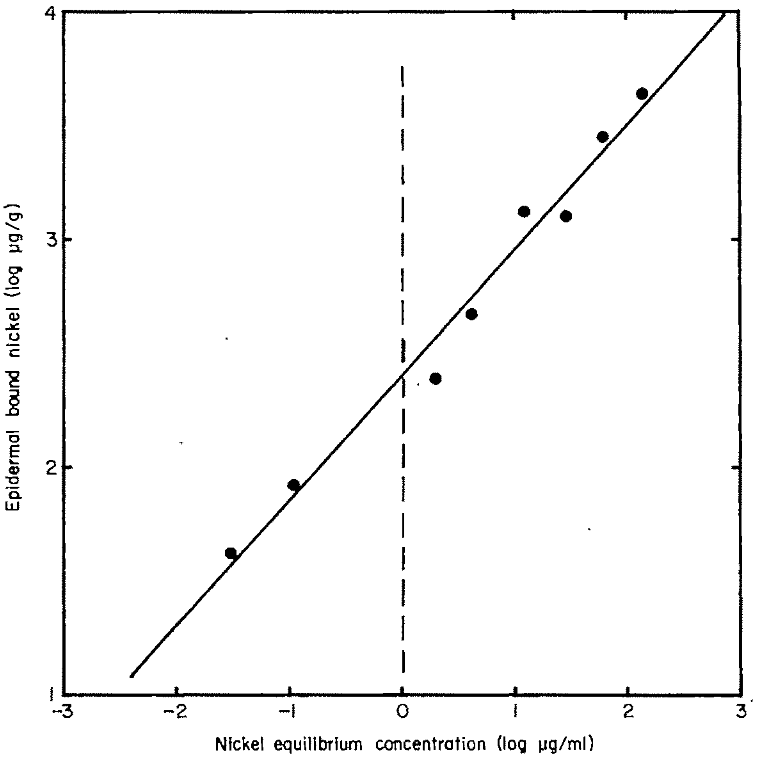

FIGURE 1. Freundlich adsorption isotherm plot of epidermal nickel binding.

adsorption equation. The binding characteristics of cobalt and of nickel are almost identical, although cobalt gave a slightly higher log K value, indicating a higher binding capacity of cobalt compared to nickel. When the incubation medium contained both nickel and cobalt, a lower epidermal binding capacity for both ions was found.

Effect of metal chelating agents

Dry powdered epidermis (50 mg) was incubated with three concentrations of nickel with and without the chelating agent in the incubation medium and the percentage change in the concentration of nickel measured. The results are shown in Figure 4.

Na-EDTA was found to be the most effective chelating agent since it removed about 70–90% of the nickel bound to the tissue at all three concentrations. L-histidine appeared less effective at the lower nickel concentration, but its blocking ability increased at the high nickel concentrations, removing about 70% of the nickel bound to the powdered epidermis. D-penicillamine, which has been shown to protect against the lethal effect of nickel chloride exposure[15], removed less than 20% of nickel from the powered epidermis.

DISCUSSION

Our results indicate reversible affinity binding of nickel to human epidermis. Using a similar technique Artuc *et al.*[12] found n values for 8-MOP and triamcinolone of 0·59 and 1·01,

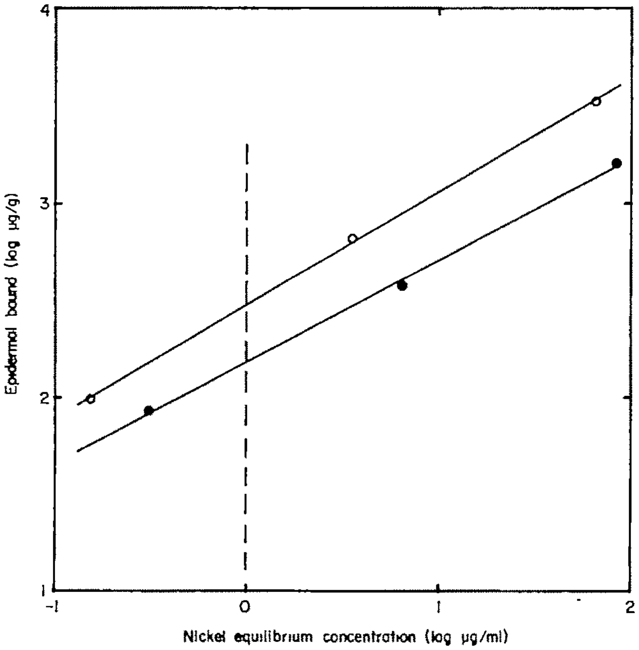

FIGURE 2. Binding of nickel to epidermis. (○) Incubation medium without cobalt; $n = 0{\cdot}58$, K = 300; (●) with cobalt $n = 0{\cdot}52$, K = 152.

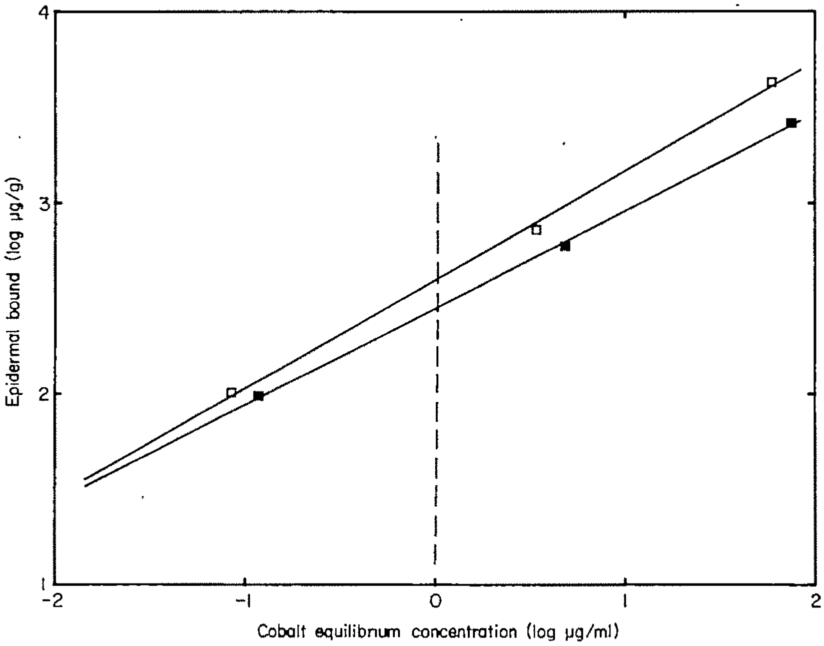

FIGURE 3. Binding of cobalt to epidermis. (□) Incubation medium without nickel $n = 0{\cdot}57$, K = 395; (■) with nickel $n = 0{\cdot}51$, K = 281.

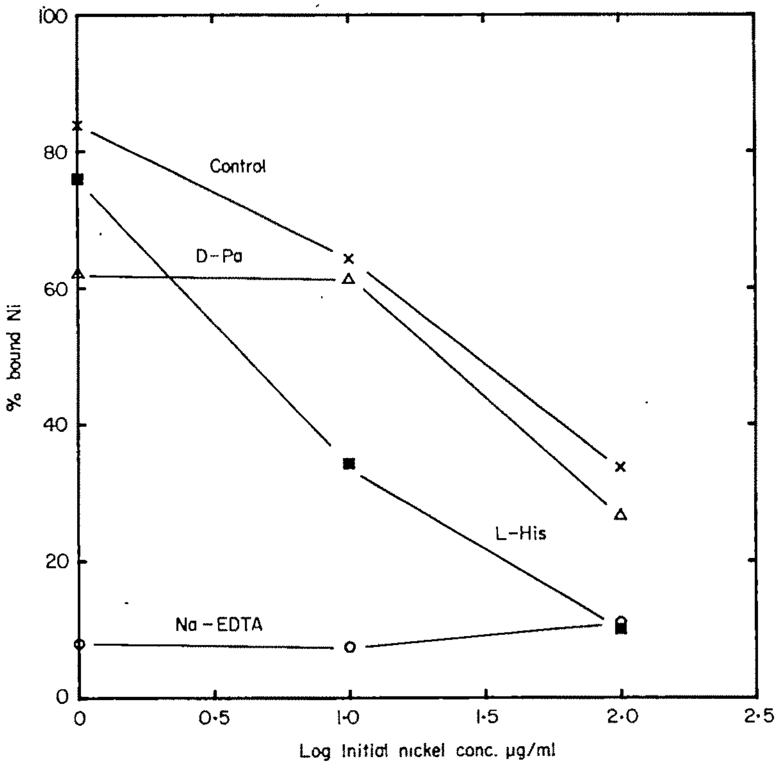

FIGURE 4. The effect of chelating agents on the binding of nickel at various initial nickel concentrations in the incubation medium ○, Na-EDTA; ■, L-histidine; △, D-penicillamine; x, control.

respectively, and proposed that 8-MOP represented reversible affinity binding while triamcinolone appeared to represent a true partitioning system.

The considerable nickel binding to epidermis is in accordance with the observation of this layer being a local reservoir.[1] The extended lag time observed in our previous studies on nickel permeation,[2,3] indicates that this epidermal binding interferes with the diffusion of nickel through the skin. However, the clinical significance of the epidermal binding observed in this *in vitro* study is speculative. In the concentration range investigated, the incubation medium with the lowest nickel concentration seems to be of primary interest. More than 90% of the available nickel was found to bind to the epidermis. Since an allergic reaction can be observed in sensitized patients within 2 days after topical application of a nickel patch test, only a small amount of nickel is needed to penetrate through the stratum corneum. Menné *et al.*[13] found allergic reaction to alloys releasing more than 0·5 µg Ni/cm^2 in 7 days to artificial sweat. If all the nickel is assumed to accumulate in epidermis at a release rate of 0·07 µg/cm^2/day, the amount of nickel in one cm^2 (corresponding to 2·8 mg dry powdered epidermis) would be at lest 50 µg Ni/g dry epidermis in 2 days. From our binding experiments, this amount of nickel would be expected to be bound to the epidermis. Therefore, it is probable that the threshold concentration of nickel producing an allergic reaction is related to the actual amount of nickel bound to epidermal tissue. When the process of nickel penetration across the stratum corneum proceeds, the concentration of nickel in the epidermis gradually increases and an increasing fraction of nickel in the epidermis is free to permeate further through the skin.

Our results showed that when the incubation medium contained cobalt in addition to nickel, both ions showed a lower epidermis binding capacity. However, if nickel and cobalt compete for the same binding sites in the epidermis, a more pronounced reduction in binding would have been expected than that which we found. The similarity in the atomic numbers and molecular weights of the two metals may explain the similarity of their binding characteristics, but the lack of significant competition for binding sites is surprising. The fact that nickel does not seem to cross-sensitize with cobalt[14] may, therefore, be attributed to the two metals occupying different binding sites in epidermal tissue. We found that disodium-EDTA was the most effective of the chelating agents in removing nickel bound to powdered epidermis, and D-penicillamine the least effective. Corresponding results for nickel binding to human albumin were reported by Reiss *et al.*[16] They found that Na-EDTA was able to remove 90% of the nickel from the binding sites, whereas L-histidine was less efficient and D-penicillamine did not remove nickel from albumin at all.

In the past, some metal chelating materials have been investigated in an attempt to demonstrate a blocking effect on patch test reactions with nickel salts.[17–20] Of particular interest is the effect on Na-EDTA. Ketal and Bruynzeel[18,19] found that the blocking effect of a 10% EDTA cream appeared to be significantly greater than that of the cream base only, when the patients were pre-treated with the cream nickel exposure. However, our present experiments were not designed to study a protection against contact allergy to nickel, but to find agents which block nickel from the binding sites in the epidermis. An effect of chelating agents on patch test reactions can be attributed either to a reduced penetration of the nickel chelate into epidermis or to reduced binding of nickel to proteins in the skin. Further investigations of this mechanism are, therefore, in progress.

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British Journal of Dermatology (1988) **119**, 683–685.

Correspondence

Fluconazole in the management of patients with chronic mucocutaneous candidosis

SIR, The management of patients with chronic mucocutaneous candidosis (CMCC) was improved dramatically with the introduction of ketoconazole, which produced cures or remissions in a significant number of those treated.[1] However, relapse of the infection occurs with increasing frequency after the initial remission and this has prompted some clinicians to use continuous prophylaxis with oral ketoconazole to prevent long-term sequelae of infection, such as the development of oesophageal strictures. Although there are few confirmed reports, azole-resistant *Candida albicans* have been isolated from some CMCC patients on continuous ketoconazole therapy.[2] Other patients become clinically refractory to the drug.

We would like to report our experience with the new oral triazole antifungal, fluconazole, in patients with chronic mucocutaneous candidosis. The drug is currently being assessed in superficial fungal infections and has been reported to be effective in both oropharyngeal candidosis[3] and cryptococcal meningitis[4] in patients with AIDS. Fluconazole is well absorbed after oral administration and shows low serum binding. It can be detected in significant levels in saliva as well as in urine and cerebrospinal fluid. It is largely eliminated via the kidneys—an unusual feature for an azole drug.

Eight patients with mycologically confirmed oral candidosis of the tongue and oral mucosa were treated with fluconazole; none had received antifungal therapy for at least 6 weeks prior to treatment with fluconazole. The seven patients had either chronic (3) or relapsing (5) oral infection associated with CMCC and had received ketoconazole as the main treatment. Those with relapsing disease had reported at least six episodes of infection within the preceding year. The chronically infected patients had been refractory, to ketoconazole prior to entering the study, but the organism had not shown resistance to ketoconazole *in vitro*. The mean age of these patients was 23 years (range 16–28); five had idiopathic CMCC and three the *Candida* endocrinopathy syndrome (hypoparathyroidism/hypoadrenalism one case and two with hypothyroidism). Each patient received 50 mg fluconazole daily (Pfizer Central Research, U.K.) for up to 4 weeks and were assessed clinically and mycologically each week by both microscopy and culture. Clinical and mycological remissions were induced in all eight patients in a mean period of 10 days (range 7–21 days). Three patients relapsed within 4 months (mean 56 days), but all responded to a further short course of oral fluconazole, 50 mg daily for three days. The frequency of relapse, however, was less than before and all four patients who were no longer responding to ketoconazole cleared on fluconazole. No patient reported any adverse reactions to the drug and no abnormality of biochemical values was detected during treatment.

We suggest that intermittent oral antifungal therapy is the most appropriate method, at present, of managing patients with CMCC after induction of remission. The rapid response rate seen with fluconazole would make this a highly suitable drug for use in such cases, although the value of this drug compared to alternatives such as ketoconazole and itraconazole needs to be assessed. Similar rapid responses of recalcitrant oral candida infections have been reported recently in patients with AIDS[3] and neutropenic cancer patients[5] receiving fluconazole.

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Foetal psoriasis

SIR, A woman of 30 who had been attending the hospital for 18 years with widespread psoriasis and nail pitting brought her 3-week-old baby to the Dermatology Department for assessment. She stated that she had noted pitting of her baby's nails when he was 1-week-old. Her psoriasis became much worse during the middle months of her pregnancy. The baby's elder brother was free from skin disease. Examination showed pitting towards the distal ends of several nails.

It is tempting to speculate that the pits in the baby's nails were a manifestation of psoriasis, and if so they must have been present in-utero.

According to Samman[1] nail pits are formed during the process of keratinization which starts in the 3rd month of embryonic life[2]. This was about the time that the mother's psoriasis became much worse. Perhaps a blood borne factor[3] passed via the placenta from mother to foetus at that time.

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Permanent cure of hydroa vacciniforme after chemotherapy for Hodgkin's disease

SIR, We should like to report a remarkable case of complete recovery from hydroa vacciniforme with chemotherapy for Hodgkin's disease. The patient, a 47-year-old man, had suffered from severe hydroa vacciniforme since the age of 19. He noted itching and burning sensations with subsequent blister formation on his face, ears and hands after exposure to sunlight. The skin lesions healed with superficial scars within about 2 weeks. Porphyria was excluded by measurements of porphyrins and precursors in urine, plasma and erythrocytes, which were within the normal ranges. Histology of skin biopsies did not show the changes characteristic of the cutaneous lesions of porphyria, but showed non-specific morphology compatible with hydroa vacciniforme. Various treatments, including topical sunscreens, oral beta-carotene and hardening with photochemotherapy did not substantially alter his light sensitivity.

In 1982 enlargement of cervical lympho nodes was observed and diagnosis of Hodgkin's disease was made on biopsy. Because the patient refused staging laparotomy and because of the clear presence of B symptoms (fever, night sweats and weight-loss) we decided to treat him with chemotherapy consisting of a C-MOPP regimen (cyclophosphamide, vincristine, procarbacine, prednisolone). After two cycles of chemotherapy the patient noticed improvement of his light sensitivity and when treatment was changed to ABVD (adriamycin, bleomycin, vinblastine, dacarbacine) because of progression of the Hodgkin's disease during C-MOPP therapy, the light sensitivity disappeared completely. After six cycles of ABVC in 1983 there was a complete remission of the Hodgkin's disease. The patient was then monitored every 4 to 6 months, and during the following 5 years he never experienced an attack of hydroa vacciniforme, and was able to tolerate normal sun exposure.

Hydroa vacciniforme is a rare disorder and the occurrence with Hodgkin's disease must be even rarer; to our knowledge no such case has been reported previously. It is known that hydroa vacciniforme may sometimes show spontaneous remission usually by late puberty. Spontaneous remission of adult cases has not been reported to our knowledge. We would speculate, therefore, that cytotoxic drug therapy might influence hydroa vacciniforme. Whether this reflects an effect of cytotoxic drugs in general or is specific to the vinca alkaloids, which were only substances used in both regimens, in our patient, remains to be determined. We think it is worth considering whether the temporary discomfort caused by cytotoxic chemotherapy and the associated possibility of an increased risk of neoplasias might be justified in patients with disfiguring hydroa vacciniforme. Perhaps low dose treatment might suffice to control this dermatosis in the severely affected patient suffering from significant physical and emotional distress.

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British Journal of Dermatology (1988) **119**, 686–689.

Book Reviews

Melanoma and Naevi: Incidence, Interrelationships and Implications Edited by J.M. Elwood (1988) Pigment Cell Volume 9; Series Editor R.M. Mackie, Basel, Karger. Pp. 156 Price £56.90.

Why does malignant melanoma continue to attract a lot of attention in the United Kingdom even though a general practitioner, with an average size of practice, might only expect to see one case every 3 or 4 years? Two reasons stand out. Firstly, epidemiologists and clinicians have demonstrated a steady increase in the incidence of the tumour in whites throughout the world so that melanoma is now runner up tó lung cancer in women in the league of cancers with an increasing incidence. Secondly, many are beginning to appreciate that it is not just a pipe dream of melanoma experts that deaths from this tumour are preventable.

Surgical gains, except for the realization that narrow (1 cm) excision of tumours of less than 1 mm thick is good rather than bad treatment, have been few and chemotherapeutic advances negligible. However, professional and public education campaigns to warn those at risk of developing melanoma and to recognize and treat the tumour early look promising and should reduce the death rate due to melanoma. Melanocytic naevi, including congenital, dysplastic and multiple banal naevi, are among the most important factors which have been identified in individuals with a high risk of melanoma.

Melanoma and naevi provides a comprehensive, yet very readable (forgiving 'photocatamnestical' to describe previous photography) account of the complex relationship between these benign and malignant tumours of melanocytes. Many of the familiar melanoma names are contributors and many of their contributions are familiar, but it is useful to have their views stitched together in a single tome.

Pigmented lesion clinics are here to stay. Those who run them should get this short book even though it is expensive; it spells out the implications that relate naevi to melanoma. It gives advice on those naevi which should be excized. It also provides helpful and objective information on those patients with naevi who should be followed up regularly by a pigmented lesion specialist, and, just as important, on those who may reasonably be asked to keep an eye on their own moles.

J.A.A. Hunter

Surgery of Basal Cell Carcinoma of the Face. *D. Marchac* (1988). Translated from the French by E. Pugash. Berlin: Springer. Pp 115. Price DM158.

It is a sign of the change in medical mores that a dermatology journal has been asked to review a book on the surgical treatment of basal cell carcinoma written by a French plastic surgeon. This useful book covers in some detail methods of wound closure after BCC excision. If you have decided that primary excision and closure is the treatment of choice for your patient then this book would be a good place to start from. Although there are 7 chapters only 14 pages are devoted to alternative therapies, surgical principles, indications and complications. The remaining 83 pages are given over to techniques of surgical repair, examples of repairs at different facial sites and a summary of results. Each section is well-illustrated with good quality black and white photographs, and at least 60% of the techniques described could be done by a surgically orientated dermatologist. I would disagree with the author's comments about histological control and am a little concerned that in the search for cosmetic excellence he sometimes takes inadequate margins (Fig 4.32). The principal strength of the book lies in the illustrated examples of closures at different sites. The shortcomings are the lack of practical tips, and insufficient examples of complications and how to avoid them.

C. Lawrence

Photodermatologie et Photothérapie—P. Thomas and P. Amblard (1987). Paris. Masson. Price 250 Francs.

I particularly enjoyed this book and I do so wish I had had it when I first started having a PUVA unit. It manages very nicely to be both short and comprehensive and gives very good explanations of the conditions for which phototherapy can be used. Each chapter has at the end a bibliography so that one can read further on any topic mentioned. The authors are modern and didactic and the book would be very suitable for Registrars who want to get an overall view of the field. Anyone starting a Dermatology unit would do well to have this friendly and helpful little book which combines succinct information with good photographs and sensible advice and it is really very up-to-date.

G. ALEXANDER

Papillomaviruses and Human Disease Edited by K. SYRJÄNEN, L. GISSMAN AND L.G. KOSS (1987) Berlin: Springer. Pp 518. Dm. 168.

The recent explosion in knowledge about papillomaviruses (PVs), due both to the recognized association with dysplastic epithelial change and the application of molecular biological techniques, has made it difficult for the busy clinician to keep pace. This multi-author text should provide the necessary update.

Essential background information on the virological properties of the PVs with straightforward descriptions of the major techniques used in their study are found in the early chapters, preceding the clinical aspects of human papillomavirus (HPV) infection. Genital dysplasia is dealt in detail, thankfully with a clear explanation of the classification of genital neoplasia. The immune response to PV infection is covered, but this area needs further elucidation before such a chapter becomes very easy reading. The more experimental aspects of PVs (cell culture systems, the state of the PV DNA in infected cells, gene expression and malignant change) form the basis for the last few chapters, and of necessity, often relate mainly to animal rather than human HPV types.

All chapters are supported with full references, often including work published in 1987 as might be expected from authors prominent in the field of PV research. The layout makes it easy to use the book either for reference or for perusal, and I would recommend it both to those working with PV-induced disease or who have already an interest in the field.

JANE C. STERLING

Transdermal Delivery of Drugs. Edited by A.F. KYDONIEUS and B. BERNER (1987). Boca Raton: C.R.C. Press. 3 vols. Pp 204 + 154 + 160. Price £284.00.

This 27 chapter, multi-author, three volume series on *Transdermal Delivery of Drugs* comprehensively covers a new and expanding area of interest in dermatopharmacology. It is only in the last 10 years or so that the possibilities of delivering systemic drugs via the transdermal route have really been explored. Prior to this, the transdermal systemic effects of drugs were of more toxicological than therapeutic interest.

Although these volumes are necessarily technical and specialist in nature, they contain much more than simply details of the pharmacodynamics and pharmacokinetics of the various transdermal delivery systems so far developed. There is also much general information about *in vitro* and *in vivo* models for skin penetration and many other aspects of skin permeability.

The first volume concentrates on the transdermal device and there is a good introductory chapter as well as an overview on the various types of transdermal delivery systems that are currently employed. There are also chapters on the kinetics of skin permeation and on the techniques used to determine *in vitro* and *in vivo* skin permeation. Many sections contain quite detailed mathematics/physics, but this is usually clearly set out and in a form that clinicians will find at least accessible if not always comprehensible.

The second volume deals with the parameters affecting skin penetration, the pharmacokinetics of transdermal delivery, penetration enhancers, cutaneous toxicology and experimental skin models and models of skin permeability.

The third volume deals mainly with the drug parameters important to transdermal delivery. There is also a useful chapter on the concept of pro-drugs and a comprehensive review on the penetration characteristics of many drugs applied topically.

Obviously, in a multi-author book such as this, there is some repetition but the volumes generally have been well produced. In so rapidly changing a field it is perhaps disappointing that the manuscript is already 4 years out of date.

At £284 for the set, it would have been nice to have been able to recommend just one of these volumes to the dermatologist but, unfortunately, there are useful chapters scattered throughout the three volumes which, even for those not primarily interested in transdermal delivery systems, provide a wealth of information on many aspects of skin penetration, permeability and pharmacodynamics/pharmacokinetics.

J.D. WILKINSON

ABC of Dermatology. P. K. BUXTON. With contributions from D.J. Gawkrodger, D.W.S. Harris, D. Kemmett, A.L. Wright. (1988). London: British Medical Association. Pp 91. Price £10.95.

This new guide to Dermatology is a collection of articles written for the B.M.J. by Dr Buxton and his colleagues. I enjoyed the original articles but the complete volume is a gem. The Edinburgh School is known for its didactic teaching of Dermatology and the approach to the subject is clear and comprehensive without delving too deeply into small print, although all the common conditions and many of the rarities are included.

Key conditions are discussed and then used as a basis for comparison with other skin disorders. The text is interspersed with diagrams and, on the whole, excellent clinical colour photographs, together with blocks indicating key teaching points. Subjects are succinctly dealt with and there is a timely article on 'Black Spots in the Skin' which may help the reader to sort out those conditions which require specialist opinion. There is also a chapter on Practical Procedures, which could be of considerable help to doctors in practice who wish to undertake such procedures in their clinics. In this type of book references are probably unnecessary, but advice on further reading is helpful.

The book is aimed at providing the non-specialist with a practical guide to diagnosis and treatment of skin conditions. In this group one should include general practitioners and other hospital doctors, but it may well complement teaching of undergraduates and junior training grades in hospital dermatology and at its price of £10.95 is a bargain.

D.M. WILLIAMSON

Encyclopedia of Conditioning Rinse Ingredients. A.L.L. HUNTING (1987) London: Micelle Press, (Wellington Home, Eltham SE9 5DP) Pp 512 Price £65.00.

It is fashionable to use conditioner on the hair after washing and this companion volume to the Encyclopedia of Shampoo Ingredients, 1983, lists 451 ingredients in 259 conditioning rinses. With it the dermatologists in America can easily find the formula of a particular marketed product if adverse reactions are suspected, and can give to allergic patients, lists of all the products that contain their allergens.

Very full physical and chemical data of all ingredients are given, reason for their use, and safety data; all in simple terms, easily understandable by those engaged in patch testing. Like all good encyclopedias it can not be put down after looking up one reference, the eye is drawn further to not immediately relevant, but fascinating bits of information.

Since April 1977 under F.D.A. regulations it has been mandatory for all cosmetics and toiletries sold, except professional products and soap, to carry a list of their ingredients and this volume is a most useful addition to this labelling. Let us hope it is not too long before we follow the U.S.A. in their commitment to freedom of information to both professionals and patients.

C. F. ALLENBY

Books Received

A Colour Atlas of AIDS and HIV Disease. C.F. FARTHING, S.E. BROWN and R.C.D. STAUGHTON. 2nd edition (1988) London: Wolfe Medical Publications. Pp 115. Price £16.00.

Handbook of Phlebotomy. G.E. PENDERGRAPH. 2nd edition (1988) Philadelphia: Lea and Febiger. Pp 135.

A Colour Atlas of the Nail in Clinical Diagnosis. D.W. BEAVEN and S.E. BROOKS. (1988) Paperback edition (hardback edition 1984 reviewed June 1985) London: Wolfe Medical Publications. Pp 240. Price £11.95.

British Journal of Dermatology (1988) **119**, 690.

News and Notices

NATO advanced Study Institute on

Skin Pharmacology and Toxicology—Recent advances **Riva del Garda (Italy) April 9–19, 1989**

A NATO Advanced Study Institute will be held at Riva del Garda (Italy) on April 9–19, 1989 on 'Skin Pharmacology and Toxicology—recent advances'. The purpose of this Course is to examine the recent advances in the state of the art of experimental pharmacology and toxicology of the skin and how they relate to cutaneous diseases.

Directors of the Course are: Professor Corrado L. Galli (Centro di Biologia e Tossicologia Cosmetologica, University of Milan, Italy) and Professor Christopher N. Hensby (Centre International de Recherches Dermatologiques, Sophia Antipolis, France).

Organizing Committee: Mrs Daniela Galli, Dr Marina Marinovich, Dr U. Reichert.

The attendance is restricted to a maximum of 60 participants. Persons wishing to attend this course should send their condensed curriculum vitae to the Organizing Secretariat. *The deadline is November 15, 1988.*

For more information contact the Organizing Secretariat, Dr Nadia Bauer, Centro di Biologia e Tossicologia Cosmetologica, University of Milan, via Balzaretti, 9-20133 Milan (Italy). Tel. 02-29404672.

British Dermatological Surgery Group

The British Dermatological Surgery Group's Sixth Annual Practical Workshop will be held on Thursday 6 and Friday 7 April 1989 at the University Hospital of Wales, Cardiff. The course will include practical instruction on all aspects of basic skin surgery. For further information please contact Dr Peter Holt, Department of Dermatology, University Hospital of Wales, Heath Park, Cardiff CF4 4XW, U.K. (0222 755944 Ext. 3167).

North of England Dermatological Society
R. Summerly Prize

The prize this year was awarded to Dr R. H. Graham for his presentation on Oro-facial granulomatosis and its relationship to Crohn's disease.

European Academy of Dermatology and Venereology 1st Congress

The 1st Congress of the European Academy of Dermatology and Venereology (EADV) will be held in Florence, Italy, September 25–28, 1989. Topics will include AIDS and the Dermatologists, What's New in Therapy?, Dermatological Surgery, Warts and Viruses, Cutaneous Histopathology and Immunopathology and Contact Dermatitis.

For further information please contact: EADV/CSS, Via Lapini, n. 1-50136 Florence, Italy.

British Journal of Dermatology (1988) **119**, 691–696.

311 nm UVB phototherapy—an effective treatment for psoriasis

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SUMMARY

Fifty two psoriatic patients were treated with a new experimental fluorescent lamp (Philips TL-01) emitting a narrow band at 311 ± 2 nm (UVB) which had the advantage of a reduction in burning and carcinogenic wavelengths when compared with conventional broad band UVB therapy. Results of the '311' treated group when compared with broad band UVB therapy revealed a similar percentage of patients achieving a satisfactory response with fewer burning episodes and an increase in duration of remission.

Phototherapy with UVB and psoralen photochemotherapy (PUVA) are well established treatment modalities for psoriasis.[1–3] Initially, both may give equally satisfactory results,[4–6] but the duration of remission obtained with PUVA is superior to that obtained with UVB.[4] PUVA is contraindicated in pregnancy, and some patients may experience severe nausea or pruritus, especially with 8-methoxypsoralen (8-MOP).[7] The main concern lies with its potential carcinogenicity; both 8-MOP and 5-MOP plus UVA are known to be carcinogenic in animals.[8] Multicentre co-operative retrospective studies in psoriatic patients treated with PUVA have produced differing results;[9–11] where an increased risk of non-melanoma skin cancer did occur, in addition to other factors such as previous treatments with arsenic or X-rays, it was related to the cumulative UVA dose and possibly also to the treatment schedules used. Until such time as derivatives such as pyridopsoralen, which theoretically have less carcinogenic potential,[12] become available for routine therapy, the use of PUVA should be restricted to severe forms of psoriasis which are resistant to topical treatment. The use of conventional broad band UVB phototherapy with a source such as the Philips TL40W/12 (waveband 280–350 nm) is often limited by burning[2] and the potential risk of carcinogenesis.[13,14] Monochromator studies by Parrish and Jaenicke[15] demonstrated that the action spectrum for phototherapy of psoriasis differs from that for UV-erythema. Wavelengths of 290 nm and below are erythemogenic but not therapeutic, even at dosages of 50 times the minimal erythema dose (MED). The therapeutic wavelengths are, therefore, > 290 nm. Moreover, Fischer *et al.* have shown that a narrow waveband at 313 nm is particularly effective for clearing psoriasis, with a reduced capacity for

Correspondence: Dr J.Ferguson, Department of Dermatology, Level 8 Polyclinic Area, Ninewells Hospital, Dundee DD1 9SY producing erythema.[16] Van Weelden and van der Leun[17] proposed that it would be desirable to develop a radiation source for UV-phototherapy which excluded the shorter erythemogenic wavelengths. Since the action spectrum for UV-carcinogenesis is thought to parallel that for erythema,[18,19] such a source would also have the theoretical advantage of being less carcinogenic than the conventional broad band source.

An experimental fluorescent lamp (Philips TL-01) has been developed with a phosphor which produces a peak narrow band emission at 311 nm (± 2 nm) and a minor peak at 305 nm (Fig. 1). Two pilot studies of the efficacy of the TL-01 lamp in the treatment of psoriasis vulgaris have been performed.[20] First, in nine patients with widespread, symmetrical psoriasis, one half of the body was treated with radiation from conventional broad band UVB using TL-12 lamps, while the other half was treated with the new TL-01 lamps, the treatment schedule being the same for each lamp type so that a slight erythema was elicited after each treatment. In the second, a comparison of whole body treatment was made in eight patients using the two types of lamps. In both studies results obtained with the TL-01 '311' lamps were significantly better than those obtained with the broad band TL-12 lamps. The authors also showed that the TL-01 lamps were significantly less carcinogenic for mice than the TL-12 lamps.

As part of an intended multi-centre trial we have studied the efficacy of these new lamps in the treatment of psoriasis in our department, comparing the results obtained with those from a trial using the conventional broad band Philips TL-12 lamps.

METHODS

During the period January to August 1986, 52 patients with extensive psoriasis (> 20% body surface area involved) were treated. In order to assess the efficacy of therapy, patients were subdivided clinically into three groups; plaque, plaque/guttate, and guttate psoriasis. Patients with a history of photo-aggravated psoriasis or previous cutaneous malignancy were excluded. Topical therapy during the study was restricted to 50:50 liquid paraffin: white soft paraffin or emulsifying ointment emollient. Phototherapy was given in an upright irradiation cabinet, 2 m high × 1·4 m × 1·2 m, containing initially 22 of the new '311' fluorescent tube lamps (Philips TL-01) arranged to provide whole body exposure with an average irradiance of 1·07 mW/cm^2 measured with a calibrated UVB meter. During the course of the study the irradiance was

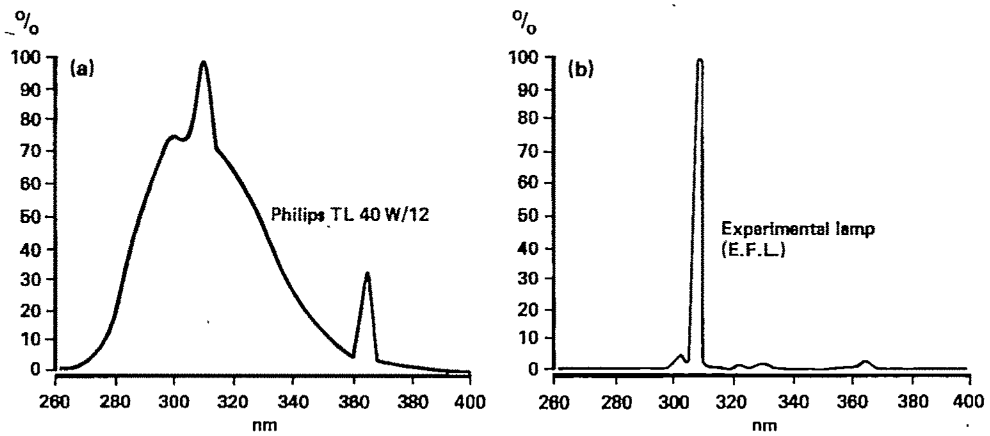

FIGURE 1. Relative spectral energy distribution of (a) the Philips TL40W/12 lamp and (b) the Phillips TL-01 lamp.

increased to 2·09 mW/cm^2 by placing reflecting foil behind the lamp fittings and by increasing the number of tubes to 49. This made it possible to reduce the treatment exposure time and so increase the availability of the cubicle to patients.

Prior to therapy, the MED for the TL-01 lamps was determined in each patient using a range of dosages established empirically during a pilot study. The final testing schedule required each patient to be seated at a distance of 20 cm from six tubes; six sites on the back, or, in a few cases where there was insufficient lesion-free skin, on the arm, were irradiated with a graded series of exposures from 50–1500 mJ/cm^2 (irradiation time 19 s–9 min 30 s). Using this schedule we found the average MED for patients with skin type II to be 500 mJ/cm^2 (range 100–1250).

Treatment was given thrice, or in five cases twice, weekly, the initial exposure dose being 70% of the MED. Subsequent exposures were increased by 40%, the aim being to achieve a mild erythema response after each treatment. The exposure schedule was adjusted if severe erythema or burning occurred, with burning being defined as severe, painful erythema (Table 1).

Patients were assessed weekly and any adverse reactions recorded. Once a satisfactory state, defined as clearing of lesions with or without minimal residual activity, was achieved, patients then received six further treatments at the same exposure dose. Once patients stopped therapy they were reviewed every 2 weeks for 8 weeks, and thereafter monthly. A relapse of psoriasis was defined as a degree of skin involvement of 50% or more of that recorded at the time of entry into the study.

RESULTS

Results of treatment in the three clinical groups are shown in Table 2. A satisfactory response was obtained in 92% of the plaque psoriasis group and in 100% of patients in the plaque/guttate and guttate groups. The mean duration of therapy (6·6 weeks) and the mean total cumulative dose (18·4 J/cm^2) were similar in each group. The incidence of burning episodes was 10%. One year after cessation of therapy, 38% of the patients remained in remission. Those patients who relapsed did so after an average of 12 weeks (range 2–52 weeks).

Comparison with conventional UVB therapy

In a previous study, 25 psoriatic patients were treated by conventional UVB phototherapy (Philips TL40W/12 lamps; irradiance 1·5 mW/cm^2) initially using the same inclusion criteria, treatment schedule and assessment criteria as in the present study.[4] However, because there were problems of burning, patients were treated twice rather than thrice weekly. Table 3 shows the comparison of results of the conventionally treated and the '311' treated groups. Both the

TABLE 1. Phototherapy schedule

Stage	
1	MED determination
2	First treatment: 70% of MED
3	Dose increments thrice weekly: If not erythema: dose increased by 40% If slight erythema: dose increased by 20%
	If more than slight erythema: same dose
	If mild burn: dose decreased by 50%
	If severe burn: dose omitted

TABLE 2. Results of UVB phototherapy with Phillips TL-01 lamps in 52 patients with psoriasis. Values are means and ranges

	Satisfactory results		Total treatment course	
Type of psoriasis	Duration of treatment (weeks)	Cumulative dose (J/cm^2)	Duration (weeks)	Cumulative dose (J/cm^2)
Plaque ($n=17$)	4·7 (2·6–9·3)	11·8 (3·4–26·0)	6·7 (5·3–12·3)	18·1 (9·9–41·5)
Guttate ($n=20$)	4·3 (1·6–7·0)	9·9 (2·6–17·8)	6·6 (3·0–9·3)	18·3 (3·8–25·6)
Plaque/guttate ($n=15$)	3·8 (2·3–5·3)	8·5 (2·2–19·8)	6·6 (4·0–11·0)	19·0 (10·6–38·1)

time taken to achieve a satisfactory response and the total duration of therapy are significantly less in the '311' treated group; the total cumulative exposure dose was of the same order of magnitude in each group. Despite the twice weekly treatment schedule, the incidence of burning in the conventionally treated group was still relatively high at 28%. In the '311' UV treated group, 38% of the patients remained in remission for a year compared with the results of conventional broad band UVB where only 5% were still in remission after this length of time.

DISCUSSION

In discussing the action spectrum for UV-therapy of psoriasis and the lamps available for this treatment, Fischer *et al.*[16] emphasized the importance of separating the burning and possible Koebner effects of the shorter wavelengths in the UVB from the therapeutic effects of the longer wavelengths around 313 nm. This approach was well supported by phototherapy action spectrum studies of Parrish and Jaenicke.[15] Moreover, a successful practical application of the hypothesis is apparent in the results of Schothorst *et al.*[21] using the Sylvania UV-6 lamp, the emission of which has a much reduced component below 310 nm compared with that of the conventional broad band UVB lamps, and of the apparently natural selective ultraviolet phototherapy at the Dead Sea.[22] Diffey and Farr have developed this concept in the form of a 'Phototherapy Index' applied to the various lamps currently used for therapy of psoriasis, the higher the index for a given end point of therapy, the less the limitation of therapy by erythema and burning.[23] The calculated index for the TL-12 lamp was 1·3 while that for the '311' TL-01 lamp was 2·8. This predicted superiority in therapeutic efficacy is borne out by the results of both the original studies by van der Leun and van Weelden[20] and by the results reported here.

We recognize the limitations of comparing results of studies which took place at different times, but still think the comparison of the previous treatment with conventional broad band

TABLE 3. Comparison of the results of '311' and conventional UVB phototherapy. Values are means and ranges

	Satisfactory results		Total treatment course	
	Duration of treatment (weeks)	Cumulative dose (J/cm^2)	Duration (weeks)	Cumulative dose (J/cm^2)
311: ($n=52$)	4·3 (2–9)	10 (3–26)	6·6 (3–12)	18 (4–42)
Conventional: TL40W 12 ($n=25$)	10·6 (3–26)	9 (7–16)	22·0 (8–43)	19 (5–41)

UVB and the narrow band '311' is valid and of interest. With the TL-12 lamps and an aggressive, thrice weekly treatment schedule, patient compliance was poor due to burning. Even with the twice weekly schedule, the incidence of burning episodes was relatively high at 28%.

In contrast, with the TL-01 lamps the incidence of burning was only 10%, allowing continued thrice weekly therapy and thereby an average total treatment course of only 6·6 weeks. Twenty-two weeks was required for the TL-12 lamps to produce the same overall mean total exposure dose of 18–19 J/cm^2 which produced a satisfactory result with both lamp types. One disadvantage of the narrow band TL-01 lamps is the relatively long exposure time required, especially when considering the reported success of less aggressive, daily exposure regimens with conventional broad band UVB sources.[24,25] However, this problem may in part be overcome by increasing the number of lamps in the cabinet. A thrice weekly schedule may be preferred to daily exposures for patients with work commitments and travelling expenses to consider. Moreover, if as reported by Fischer *et al.*[16] psoriasis is cleared with exposure doses of 0·5 MED rather than the aggressive regimen used in our study, exposure times will again be reduced.

Thus, our work has shown the '311' lamp to be at least as effective for treating psoriasis as the broad band TL-12 source, with some clinical advantages. It is of interest to put this result in the context of the work of Parrish[26] which has shown that simultaneous or subsequent exposure to shorter wavelength radiation not only lessens the therapeutic effect of longer wavelength UVB, but also may obviate it completely. Without maintenance treatment, the remission obtained with the TL40W12 lamps was disappointing, only eight patients of the 25 treated continuing to be satisfactory for longer than 8 weeks. With the TL-01 lamps, of the 38% who remained in remission for a year or more, one-third were patients in the plaque psoriasis group. Normally, we would expect treatment with PUVA to be required for such a result and although the number of subjects in the present study was small, the remission times do appear to be very similar to those obtained with PUVA.[27]

Over the past few years, knowledge that the retinoids have an anti-cancer effect[28] has stimulated workers to study these drugs in combination with both UVB[29] and PUVA.[30,31] These combinations have proved successful in the treatment of psoriasis with a significant reduction in the mean total exposure dose. The experimental finding that the new TL-01 lamp also carries less risk in terms of UV-carcinogenesis is very encouraging in itself and the study of its use in combination with one of the oral retinoids would be of considerable interest.

ACKNOWLEDGMENTS

We would like to thank Sister Margaret Bell and her nursing staff, and Mrs Davina Watson and her technical staff, for invaluable help in this study.

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Since this paper was submitted, a detailed report of the original study has been published:

Van Weelden H, Baart de la Faille H, Young E, van der Leun JC. A new development in UVB phototherapy of psoriasis. *Br J Dermatol* 1988; **119:** 11–19.

British Journal of Dermatology (1988) **119**, 697–705.

UVB phototherapy of atopic dermatitis

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SUMMARY

UVB phototherapy of atopic dermatitis was investigated in two paired-comparison studies. In study I, 17 patients were treated for 8 weeks with ultraviolet B radiation (UVB) on one half of the body and with visible light (placebo) on the other. A severity score based on eight clinical variables was determined before, during and after treatment. The total score, pruritus score and overall evaluation score were significantly better on the UVB treated sides than on the placebo treated sides ($P < 0.001$). In 13 patients the UVB treated side healed or improved considerably, while the placebo treated side improved considerably in one patient only.

In study II, 25 patients were treated for 8 weeks with 0·8 of the minimal erythema dose (MED) of UVB on one half of the body and with 0·4 MED UVB on the other. No statistically significant differences between these dosage regimens were found.

Atopic dermatitis (AD) is a troublesome disease—not only on cosmetic grounds; the pruritus, that almost invariably accompanies it, causes the patient a great deal of distress. About 3% of all infants suffer from the disease.[1] It has been widely recognized that AD tends to disappear before adulthood in the vast majority of patients. A recent Swedish study,[2] however, shows that in at least 40% of cases, AD persists into adulthood making it quite a large problem, at least in the relatively sun-poor countries of Scandinavia. Most AD patients give a history of improvement during the summer months. The reason for this has been subject to debate. In literature, especially in older papers, ultraviolet radiation (UVR) has often been claimed to be of no or only occasional benefit.[3–4]

Different treatment modalities have been advocated, but phototherapy in the management of AD has received little attention. Surprisingly few studies have been published on this subject.[5–15]

The aim of the present studies was to elucidate whether or not ultraviolet B radiation, UVB (280–315 nm), has any effect in the treatment of AD, and, if so, which doses are optimal.

We present two paired-comparison studies, one in which UVB was compared with placebo, and the other in which two UVB treatment regimens were compared with each other.

MATERIALS AND METHODS

Design of the studies

Study I: UVB versus placebo. In a bilateral randomized comparison, patients were irradiated with UVB on one side of the body and with placebo (visible light) on the other.

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Study II: UVB, 0·8 MED versus 0·4 MED. In this bilateral comparison, patients were treated with UVB on both sides of the body—one side receiving 0·8 of the minimal erythema dose (MED) assessed for that particular patient, and the other receiving 0·4 MED.

Patients

All patients treated fulfilled the criteria of atopic dermatitis laid down by Hanifin and Rajka.[16] Only mild corticosteroid preparations (hydrocortisone 0·5–1%) and emollient creams were allowed as topical treatment during and 2 weeks prior to the start of phototherapy. Patients having received phototherapy or having sun-bathed 4 weeks prior to the UV treatment were excluded. Patients who had used oral corticosteroids, who had asymmetrical AD lesions or who were aged under 15 years were also excluded.

Study I comprised 17 patients, 10 men and seven women, mean age 24·9 years, range 20–42 years. The total duration of the disease ranged from 2 to 31 years, mean 20·1 years. Originally, 28 patients entered this study; 11 dropped out. Patients' skin types are listed in Table 1.

Study II comprised 25 patients, five men and 20 women, mean age 25·9 years, range 16–59 years. The total disease duration ranged from 4 to 54 years, mean 21·4 years. Six of the 31 patients originally entering the study were excluded. Patients' skin types are listed in Table 1.

Treatment methods

The UVB equipment used was identical in both studies. It consisted of 14 Philips TL 12 40W and 14 Philips TL 12 20 W tubes arranged in a cubicle. The irradiance, measured with an International Light radiometer/Photometer IL 1350 (using IL SED 240 as UVB-probe and IL SED 015 as UVA-probe), at a distance of 40 cm was 0·85 mW/cm^2 UVB (280–315 nm) and 0·40 mW/cm^2 UVA (315–400 nm). Since this UVA output is considered to be quite small, the cubicle used was considered a 'pure' UVB source. The spectral distribution is shown in Figure 1. The placebo tubes used in study I were ordinary daylight tubes—Osram L 36 W/30—with no measurable UV content.

Each patient was evaluated before the start of the treatment and thereafter every second week until termination of the therapy. The patients were assessed for eight different variables: pruritus, lichenification, scaling, xerosis, vesiculation, excoriations, erythema and an overall evaluation. Each of these variables was assigned a score from 0 to 3; 0 = none, 1 = light,

TABLE 1. Skin types of patients participating in the two studies

	No. of patients	
Skin type	Study I	Study II
I	2	0
II	2	8
III	11	15
IV	2	2
Total	17	25

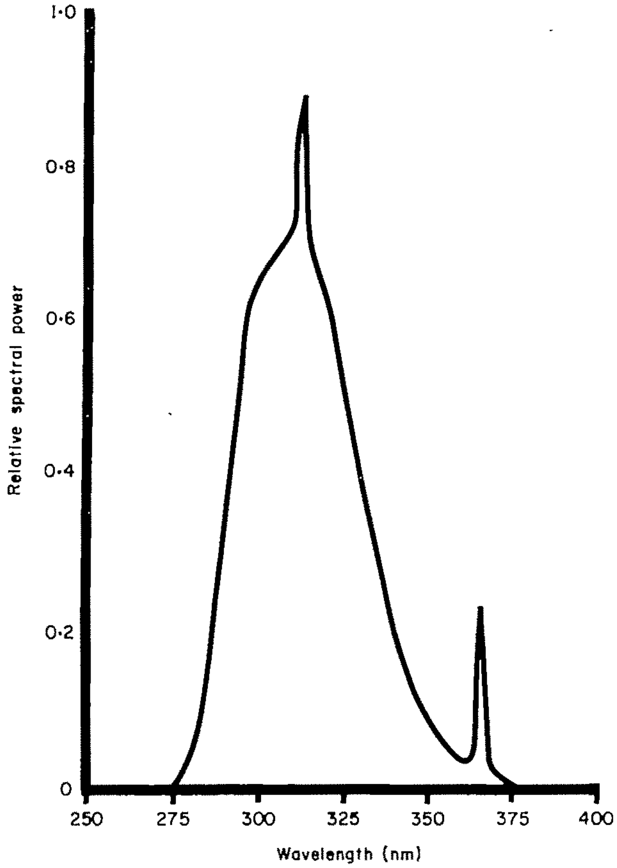

FIGURE 1. The emission spectrum for the Philips TL 12 fluorescent tubes used in the studies.

2 = moderate and 3 = severe. An assessment of healing was also made on a 5-point scale: 3 = healed, 2 = considerably improved, 1 = somewhat improved, 0 = unchanged and −1 = worsened. A body-half was considered healed if no erythema, papules, excoriations, vesicles, lichenification or scaling remained. The designation 'considerably improved' was used when a body-half was almost healed, while 'somewhat improved' designated slight to moderate improvement. Treatment of the face and the hands was not evaluated. In study I, the percentage of the skin involved was noted, and the patients were also asked to complete an evaluation form after the end of the treatment. At each visit the patients were asked whether both body-halves had received equal amounts of emollients and hydrocortisone cream or ointment.

The Wilcoxon matched pair signed rank test (two-tailed) was used for statistical analysis.

Treatment procedures

Study I. Each patient's minimal erythema dose (MED) of UVB was determined before the commencement of the phototherapy. The patients were randomized into two treatment groups—one starting with 0·5 MED and one with 1·0 MED UVB, randomized to the right or left side of the body. In the 0·5 MED group, the dose was increased by 20% each time until erythema appeared, when the dose was decreased to half of the last dose given. Thereafter, the

20% increase schedule was resumed. In the 1·0 MED group, the doses were increased in a similar manner. However, in this group no dose reduction was made at the appearance of erythema. Instead, the dose was kept unchanged until erythema was no longer seen. The 20% dose increase schedule was then resumed.

The initial doses were in the range of 20–153 mJ/cm^2 and the final doses in the range of 63–816 mJ/cm^2. The patients were given the same exposure time in the UVB cabinet as in the visible light cabinet; in both cases one side of the body was shielded with two layers of thick dark cotton sheeting.

Study II. In this study, the MED was determined every other week on the right and left body-halves separately. One side of the body was treated with 0·8 MED and the other with 0·4 MED. Dose increments were made stepwise every other week on the basis of the MED. The method of shielding the side of the body not being irradiated was similar to that employed in Study I. The initial doses on the 0·4 MED sides were in the range 7–36 mJ/cm^2, on the 0·8 MED sides 14–72 mJ/cm^2. The corresponding final doses were in the range 20–77 mJ/cm^2 and 51–173 mJ/cm^2, respectively.

In both studies, treatment was given three times a week for a maximum of 8 weeks or until the healing of at least one body-half. No treatments were given during the summer months.

RESULTS

Study I

Thirteen of the 17 patients healed or improved considerably on the UVB treated side, compared with one patient who improved considerably on the placebo side. All UVB treated body-halves achieved improvement, as shown in Table 2. The mean healing score was 1·9 after UVB treatment and 0·7 after placebo treatment; thus UVB gave a significantly better score than placebo ($P < 0{\cdot}0001$). The mean scores for the most important variables are shown in Table 3. The total score (sum of scores for all eight clinical variables) was found to be significantly lower on the UVB treated body-halves than on those treated with placebo ($P < 0{\cdot}001$). This was also the case with the pruritus score ($P < 0{\cdot}001$) and the overall evaluation score ($P < 0{\cdot}001$). The activity of the patients' dermatitis as measured by the total score improved significantly on both the UVB treated side ($P < 0{\cdot}001$) and the placebo treated side ($P = 0{\cdot}008$).

TABLE 2. Study I. Effects of UVB and placebo irradiation in 17 patients with atopic dermatitis

	No. of patients			
	UVB side		Placebo side	
Healed	3	13	0	1
Considerably improved	10		1	
Somewhat improved	4		11	
Unchanged	0		4	
Worsened	0		1	

TABLE 3. Study I. Clinical scores before treatment and after treatment with UVB and placebo in 17 patients with atopic dermatitis

	Total score*	Pruritus score	Overall evaluation score
	mean (range)	mean (range)	mean (range)
Before treatment	9·9 (6·5–19)	2·2 (1–3)	1·5 (1–3)
After UVB	5·0 (1–9)	0·8 (0–2)	0·7 (0–1·5)
After placebo	8·0 (4–13)	1·8 (0·5–3)	1·4 (0·5–2)

* Sum of scores for eight clinical variables.

TABLE 4. Study I: Patient preferences with respect to three major clinical variables

	No. of patients ($n = 17$)		
	UVB best	No. difference between UVB and visible light	Visible light best
Pruritus	12	3	2
Xerosis	3	7	7
Overall	14	2	1

Before the start of treatment the mean percentage of skin involved with dermatitis was 13·0% (range 2–89%) on the side to be treated with UVB and 13·1% (range 2–89%) on the other side. Treatment with UVB decreased this percentage to a mean of 2·5% (range 0–12%), and placebo reduced it to a mean of 7·5% (range 1–25%). UVB produced a significantly larger improvement than did placebo ($P < 0{\cdot}001$). Patient preferences are shown in Table 4; 14 of the 17 patients preferred UVB and only one visible light.

No statistically significant difference between the treatment with 0·5 and 1·0 MED could be detected, but the number of patients was relatively small for this analysis.

Ten of the 17 patients stated they had used more corticosteroids on the placebo side, while only one had used more on the UVB side. The remaining six patients had used equal amounts on both sides or used no corticosteroids at all. While the use of topical hydrocortisone tended to be increased on the placebo sides, the reverse was true for emollients; two patients used more emollient on the placebo side, four used more on the UVB side and 11 used equal amounts on both sides.

Eleven of the patients who originally entered the study withdrew. Only one was excluded because of side-effects, namely UVB-burn. The remaining 10 stopped treatment on other grounds—primarily intercurrent disease or lack of time for treatment.

The side-effects reported by the patients were tolerable and quite mild. Thirteen of the 16 patients who answered the questionnaire after completing the study stated they had experienced burning after some of the UVB treatments, but none reported burning on the placebo side ($P < 0{\cdot}0001$). Only two of these patients considered this side-effect to be severe—both were receiving 1·0 MED. Some patients complained of dryness, but there was no statistically significant difference between UVB and placebo treatment in this respect.

No reliable conclusions could be drawn from follow-up because almost all patients received whole-body UVB treatment after the completion of the study.

Study II

Fifteen of the 25 patients healed or improved considerably on the 0·8 MED side compared with 16 patients on the 0·4 MED side. Only on four and three body-halves, respectively, did the dermatitis remain unchanged or increased (Table 5). The remaining body-halves were somewhat improved. There is no statistically significant difference between the healing score results achieved by the two dosage regimens. No significant differences in clinical scores were

TABLE 5. Study II. Effects of UVB treatment with 0·8 and 0·4 MED in 25 patients with atopic dermatitis

	No. of patients			
	0·8 MED-side		0·4 MED-side	
Healed	8	15	9	16
Considerably improved	7		7	
Somewhat improved	6		6	
Unchanged	1		0	
Worsened	3		3	

TABLE 6. Study II. Clinical scores before treatment and after treatment with UVB 0·8 MED and 0·4 MED in 25 patients with atopic dermatitis

	Total score*		Pruritus score		Overall evaluation score	
	mean	(range)	mean	(range)	mean	(range)
Before treatment	10·7	(6–19)	2·4	(1–3)	1·6	(1–3)
After 0·8 MED	7·0	(0–21)	1·2	(0–3)	1·1	(0–3)
After 0·4 MED	6·6	(0–21)	1·2	(0–3)	1·0	(0–3)

* Sum of scores for eight clinical variables.

found between the two treatments (Table 6). There was a statistically significant improvement in the total clinical score on both sides ($P < 0{\cdot}01$).

Six of the patients entering the study were excluded. One of these had a very severe AD and could not manage without the more potent corticosteroids. One patient experienced troublesome UVB-burn and one experienced no benefit from the treatment. The remaining three stopped treatment owing to lack of time. All these six patients left the study within the first 4 weeks.

The side-effects reported were similar to those reported in study I.

DISCUSSION

The results of our studies showed UVB to be superior to placebo in the treatment of AD. UVB induced improvement and helped to control pruritus. The UVB dose given, provided it was held within certain limits, seemed to be of no importance. Many patients find UVB therapy relatively convenient, considering that they—while healing—can use less ointments, and the treatment is cosmetically appealing, giving them a certain tan. It is considered to be relatively safe.[17] Disadvantages include UVB erythema and the necessity for treatment to be conducted on a regular basis.

UVR therapy has not been employed to any great extent for the treatment of AD in, for example, the U.S.A.[18] The discussion has mainly circled around PUVA therapy, which has been shown to be effective.[5,8,10,12-15] The hazards of PUVA in long-term therapy have, however, made it less attractive in the treatment of this chronic disease affecting mainly young people. Also UVA therapy,[11] especially in combination with UVB,[6,7,9,13] has, in uncontrolled studies, been shown to be beneficial.

To our knowledge, our study I is the first one in which UVB therapy has been compared with placebo treatment in a matched manner. Our results are in accordance with three of the previously published reports,[6,7,9] although they are in discordance with those of one small study, by Morison *et al.*[10]

In our study a statistically significant improvement was achieved also with placebo, daylight, tubes. We consider this to be attributable to improved motivation of the patients while participating in the study, resulting in more regular use of emollients and mild corticosteroids. An increased feeling of well-being which might be expected when the patient sees his doctor frequently may also have played a role.

The difference between UVB and placebo might in reality be even greater than we recorded, since the use of topical corticosteroids was less on the UVB treated body-halves.

Our study also indicates that the systemic effect of UVR—if any—seems to be of little clinical importance. These findings are in accordance with the studies of Morison *et al.*[10] and Toews *et al.*[19] Some evidence of systemic immunological manifestations of PUVA, however, does exist.[15]

The relatively large number of withdrawals in study I is somewhat disturbing, but may—in part—be explained by the fact, that the study was performed at a day-care centre, where patients can receive phototherapy without making an appointment. These patients are less likely to feel committed to attend for treatment, than are those who have made an appointment.

In atopic dermatitis a number of immunological defects are known to occur, some of which have been ascribed pathogenetic importance. A relative T cell deficiency—particularly affecting the T suppressor cell population—has been demonstrated.[20,21] There is also an impairment in the function of the T cells.[20,22] Zachary *et al.* have shown a predominance of T helper cells admixed with Langerhans cells in the dermal infiltrate in AD, suggesting that type IV hypersensitivity may be implicated in the aetiology of the disease.[23] Evidence of type I hypersensitivity, however, also exists.[24] Furthermore, an increased number of mast cells has been show in lichenified AD.[25,26]

The mechanism by which UVR exerts its action in AD is not fully understood, even though we do have a number of clues in this respect. UVR has been shown to induce proliferation of UV-resistant epidermal cells, which stimulate T suppressor cell function.[27] This mechanism is thought to be involved in the UVR-induced non-responsiveness to contact allergens.[19] UVR also causes a decrease in the activity of the epidermal cell derived thymocyte activation factor (ETAF),[27,28] a factor which may be identical to interleukin 1. This substance is known to stimulate activated T helper cells. Apart from these suppression-promoting effects, UVR also has effects on the Langerhans cells (LC). It produces a reduction in the numbers of LC as well as an impairment of antigen-presenting function.[19,29] The itch and flare and flare response caused by the histamine-liberating agent 48/80 is decreased and the degranulation of mast cells is reduced after exposure to moderate doses of UVR.[30]

The skin has been shown to be colonized by *Staphylococcus aureus* in a great many cases of AD.[31] The role of this organism as well as that of *Pityrosporon orbiculare* in the pathogenesis of AD has been a matter of debate.[32] In a recent study, UVB has been shown to have excellent antimicrobial properties, producing inhibition of the growth of *P. orbiculare* particularly, but also of *S. aureus*.[33]

All of these actions of UVR are beneficial when treating AD and we believe that they constitute the basis for the favourable results obtained in our studies.

Thus, our studies have shown irradiation with UVB three times a week to be effective in the treatment of atopic dermatitis. Doses of 0·4 MED are as effective as those of 0·8 MED, but give fewer side-effects. On the other hand, a low-dose regimen is more difficult to carry out in clinical practice, where it is not practical to measure the MED on a regular basis, thus risking irradiation at too low a dose.

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Elevated levels of interleukin 2 receptor in sera of patients with atopic dermatitis and psoriasis

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SUMMARY

Elevated levels of serum interleukin 2 receptor (IL2R) represent an early measure of T cell activation. The concentration of IL2R was measured in the sera of patients with atopic dermatitis ($n = 58$) or psoriasis, without psoriatic arthritis, ($n = 26$), who had moderate to severe disease activity, and in non-atopic healthy controls ($n = 37$) by an ELISA technique. Serum IL2R levels were found to be significantly elevated in both disease groups compared with the controls. The increase in serum IL2R may be due to T cell activation in the dermis.

Interleukin 2 plays an essential role in triggering proliferation of activated T cells. The response is mediated by binding of the cytokine to a high-affinity membrane receptor.[1] Synthesis and expression of this receptor, which has been shown to be released from T cells and a subpopulation of B cells upon activation, represent a very early sign of T cell activation.[2–4] Increased levels of interleukin 2 receptor (IL2R) in serum have been detected in different diseases accompanied with T cell activation and changes in the immune system, e.g. rheumatoid arthritis, lupus erythematosus, T cell leukaemia, allograft rejection and the acquired immunodeficiency syndrome.[5–8]

Atopic dermatitis (AD) and psoriasis represent inflammatory skin disorders which are characterized by significant changes in humoral and cellular immunity, particularly exhibiting alterations in T cell-related functions.[9–16] In the present study we, therefore, investigated, whether T cell activation in the skin results in measurable changes in IL2R in the serum of patients with moderate to severe disease activity.

METHODS

Subjects

The atopic dermatitis (AD) group consisted of 58 patients (29 male, 29 female; age range 15–82

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years, mean age 28 years) with moderate to severe disease activity: ⩾25% of the body surface area was involved and all patients had excoriated skin lesions with intensive pruritus. The diagnosis was established according to the criteria of Hanifin and Rajka.[17] The psoriasis group consisted of 26 patients (19 male, seven female; age range 17–77 years, mean age 46 years), with extensive plaque-type psoriasis involving ⩾25% of the body surface area. None of the psoriasis patients suffered from psoriatic arthritis. The control group consisted of 37 healthy blood donors (27 male, 10 female; age range 18–59 years, mean age 29 years). Atopy was excluded by history and laboratory findings (measurement of total IgE and specific IgE to timothy grass, house dust mites, food allergens and moulds, and a differential blood count). Neither patients nor controls had received any medication (particularly local or systemic steroid therapy, Goeckerman treatment or therapy with ultraviolet light) for at least 3 weeks prior to blood sampling. Serum samples were immediately frozen and stored at −70°C.

Measurement of interleukin 2 receptor in sera

Serum IL2R was measured by an ELISA technique using the Cellfree® interleukin 2 receptor test kit (T cell sciences, Cambridge, MA, U.S.A.) in serum samples from patients and controls.[2,3] All determinations were carried out using ELISA kits with the same lot number.

Statistical analysis

Statistical analysis was performed using the Mann-Whitney U-test. For measurement of correlations IL2R levels and age, Spearman's rank correlation coefficient was used.

RESULTS

Serum level of IL2R were significantly higher in patients with atopic dermatitis and those with psoriasis, compared with the controls ($P < 0.001$) (Fig. 1). Mean IL2R levels (± SEM) were 554·9 ± 51·1 U/ml, 614·8 ± 78·3 U/ml and 312·1 ± 17·3 U/ml in the AD patients, psoriatic patients and controls, respectively.

There were no significant differences between IL2R levels in males and females in any of the three groups, or when subjects in all three groups were analysed together. Mean IL2R levels (± SEM) were 467·8 ± 41·2 U/ml for male AD patients and 642·1 ± 91·5 U/ml for females, 627·8 ± 98·7 U/ml and 579·6 ± 123·5 U/ml for male and female psoriatic patients, respectively, and 304·5 ± 17·2 U/ml and 332·7 ± 45·7 U/ml for male and female controls, respectively.

Analysing all subjects together, there was no significant correlation between serum IL2R levels and age ($r_s = 0.085$). When the three groups were analysed separately, there was a significant correlation between serum IL2R levels and age in the psoriasis group ($r_s = 0.605$; P 0·005), but no significant correlation in the AD and control groups ($r_s = -0.029$ and -0.244 respectively).

DISCUSSION

The results show that sera of patients with active AD or psoriasis contained significantly elevated concentrations of IL2 receptor. Disturbance of T cell functions may contribute to the pathogenesis of both these inflammatory skin disorders.[18,19] However, in lymphoproliferative diseases, in AIDS patients, or during allograft rejection the increase in serum IL2R levels appears to be more pronounced, for example levels 10-fold higher than in normals have been reported in Hodgkin's disease.[5–8] In contrast to these disorders the inflammatory process in AD

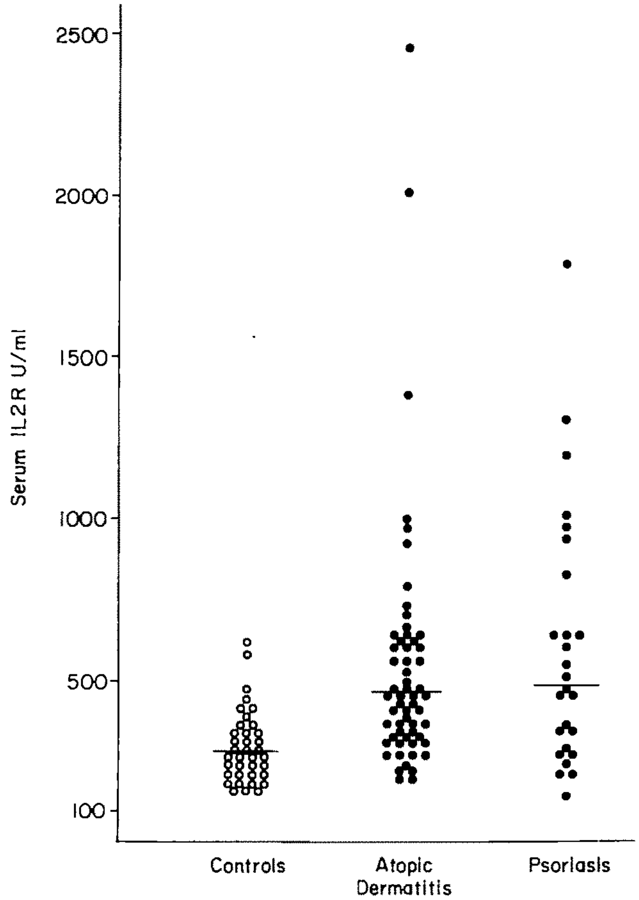

FIGURE 1. Serum levels of interleukin 2 receptor in patients with atopic dermatitis or psoriasis and in non-atopic healthy controls. Bars indicate medians.

and psoriasis is localized in the skin. Measurement of immune functions and immunomodulatory mediators in the peripheral blood only reflects the situation in the dermis. With respect to increased IL2R levels, no hyperproliferation of peripheral blood lymphocytes has been observed in AD and psoriasis *in vitro*; lymphoproliferative responses upon stimulation have been found to be normal or even decreased in both diseases.[14–16, 18–19] Decreased lymphoproliferation, however, may possibly be due to down-regulation of T cell responses in the periperhal blood by immunomodulatory mediators produced by hyperactivated immune cells in the dermis. Another explanation could be that circulating mononuclear cells are 'exhausted' after previous hyperactivation.

Based on our findings, an increase in serum IL2R is not specific to AD or psoriasis. However, T cell activation is evident in both diseases. Whether T cell activation represents a primary event in the pathogenesis of AD and psoriasis is unclear at present. Interestingly, exacerbation of AD[20] as well as of psoriasis[21,22] has been reported in AIDS patients. Therefore, T cell activation may play an important role in propagation of the inflammatory response in both diseases. Further investigations are necessary to determine which stimuli trigger the T cell response in the skin.

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No detectable increase in sister chromatid exchanges in lymphocytes from mycosis fungoides patients after topical treatment with nitrogen mustard

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SUMMARY

We measured sister chromatid exchanges (SCEs) in lymphocytes of eight MF patients before, and 2 h, and 24 h after treatment with topical nitrogen mustard. The mean number of SCEs/cell (± SEM) were 8·15 (±0·62), 8·34 (+0·55) and 8·26 (±0·56), respectively. The 2 h and 24 h results do not differ significantly from pretreatment levels. Our results show that concentrations of nitrogen mustard used to control MF do not induce SCEs in peripheral blood lymphocytes.

Mycosis fungoides (MF) is a rare, chronic, malignant T lymphocyte neoplasm of the skin. In the later stages the lymph nodes and viscera may be involved and the disease usually ends fatally after a number of years.

Topical treatment with nitrogen mustard (NM) has been used in MF since 1959.[1] Topical NM has been shown to give remission rates of up to 85%.[2]

Exposure to NM has been shown to cause cancer in animals.[3] Epidemiological studies in man indicate that exposure of soldiers to NM during World War I and occupational exposure of workers may be associated with an increased risk of respiratory tract cancers.[4]

Nitrogen mustard as well as sulphur mustard are electrophilic compounds, which are known to alkylate and crosslink DNA,[5] primarily at the N_7 position of guanine.[6] In accordance with these properties, mustards have been shown to be mutagenic *in vivo*[7] and *in vitro*,[8] and can cause a number of other genotoxic effects such as unscheduled DNA synthesis, DNA replication inhibition,[9] chromosomal damage[10] and sister chromatid exchanges (SCEs).[11]

SCEs are exchanges of apparently identical segments of sister chromatids, and assay of these

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is one of the most commonly used methods for detecting DNA damage following exposure to genotoxins.[11,12]

Previously, using a standard method for detecting SCEs in lymphocytes we found that fishermen accidentally exposed to sulphur mustards from leaking gas shells exhibited elevated levels of SCEs.[13] These results showed that using the SCE assay it was possible to detect putative systemic genotoxic effects of a single exposure to mustard gas. Therefore, we decided to investigate whether topical treatment of MF patients with NM results in increased levels of SCEs. In the present study we measured SCEs in lymphocytes from peripheral blood of MF patients before and after topical treatment with NM.

METHODS

Eight patients with MF, four women and four men from a registry of MF patients at the Marselisborg Hospital were selected for the study. Patient details are shown in Table 1. Their ages ranged from 55 to 85 years (mean 70 years). MF disease stages ranged from I–IV and disease duration from 1 to 13 years.

Nitrogen mustard (Mustine) at concentrations adjusted according to body surface area (dose range 5–20 mg per treatment) was applied topically to the whole body except the neck and head. Treatment, with NM was carried out following the safety criteria by Thestrup-Pedesen *et al.*[15] No other treatment was administered to any of the patients during the 3 months before the study.

Heparinized blood was drawn immediately before the treatment (time 0), and 2 h, and 24 h after the end of treatment, and the samples were shipped to the University Hospital, Copenhagen. On arrival (24 h later), lymphocytes were isolated on a Ficoll-paque (Pharmacia) gradient, washed three times in RPMI 1640 (Flow) after which cultures were established. The culture media consisted of RPMI 1640 (Flow) supplemented with 15% heat inactivated fetal calf serum (Flow) 2% phytohaemagglutinin (Welcome) 2 mM L-glutamine (Gibco) and 0·02 mM 5-bromo-2-deoxyuridine (Sigma). Lymphocytes were added to each culture to a final concentration of 10^6 cells/ml. The cultures were incubated for 72 h in a 5% CO_2: air atmosphere

TABLE 1. Details of patients with mycosis fungoides included in the study

Patient no.	Age (years)	Sex	Disease duration (years)	Disease stage*	No. of previous treatments with nitrogen mustard
1	78	M	8	II	> 150
2	76	F	13	II	145
3	55	M	2	I	35
4	70	F	1	I–II	3
5	85	M	?	I–II	1
6	70	M	1	II	14
7	79	F	?	I	1
8	67	F	5	IVB	50

* Classification according to Molin *et al.*[14]

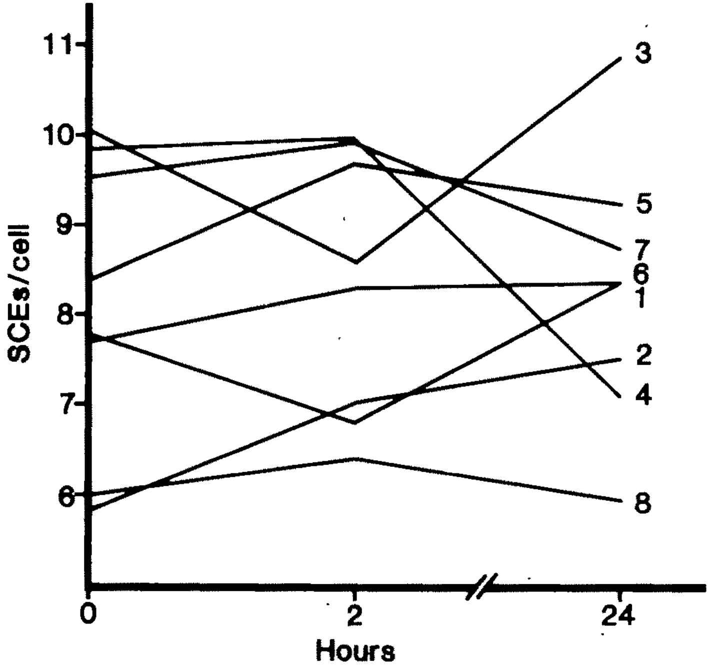

FIGURE 1. Individual sister chromatid exchange (SCE) frequencies in lymphocytes isolated from whole blood drawn immediately before, and 2 h, and 24 h after treatment with nitrogen mustard. Numbers are patient members.

in the presence of Colcemid 0·1 μg/ml (Gibco) for the last 2 h. Harvesting of the cultures and staining of the chromosomes were carried out as previously described.[16]

Evaluations of SCEs were carried out blind in 30 cells from each specimen. The results were analysed using the Wilcoxon test for paired data.

RESULTS

The individual of SCE analyses before and after treatment with NM are shown in Figure 1. The mean numbers of SCEs/cell (± SEM) before treatment, 2 h after treatment, and 24 h after treatment were 8·15 (± 0·62), 8·34 (± 0·55), and 8·26 (± 0·56), respectively. The values 2 h and 24 h after treatment did not differ significantly from the pre-treatment level.

No associations between pre-treatment SCEs or numbers of SCEs after treatment with NM and age, sex, duration of disease, or number of previous treatments could be demonstrated.

Furthermore, since tobacco smoking is known to interfere with SCE levels,[17] possible associations between smoking and numbers of SCEs before and after treatment with NM were investigated. However, no such associations were demonstrated.

DISCUSSION

Nitrogen and sulphur mustards are well known mutagens. This class of compounds induces

SCEs *in vitro*,[11] and it has been shown that accidental exposure of humans to sulphur mustard from leaking gas shells led to increased levels of lymphocyte SCEs.[13]

In contrast to these findings the present study showed no significant changes in lymphocyte SCEs following topical treatment of MF patients with NM. The SCE assay is a very sensitive assay for detecting exposure to mutagens or carcinogens.[11] Furthermore, it is known from studies on SCEs in patients treated with chemotherapy, that the lymphocyte SCE assay responds to inducing agents within 24 h after exposure.[18,19] The lack of increases on SCE levels after topical application with NM thus suggests that this treatment does not result in an increased systemic, mutational burden. Furthermore, studies showing that topical treatment of MF patients with similar concentrations of NM does not lead to bone marrow depression or any decreased immune reactivity of peripheral lymphocytes[20] provide indirect support for our findings.

Topical treatment with NM is only carried out in Denmark at the Marselisborg Hospital. Furthermore, only a limited number of MF patients were able to meet the minimum requirements for an unbiassed study of effects of NM treatment. Therefore, the present investigation included only eight individuals. Despite this our study showed no differences in SCEs or any evidence of trends towards an increase in SCEs at the dosage of NM used in topical treatment. This observation lends support to the opinion that these levels may not pose a hazard to the patients with respect to increased levels of mutation.

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A comparative immunoelectron microscopic study of typical and atypical cases of pemphigoid

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SUMMARY

Using an immunoelectron microscopic technique we have compared the ultrastructural localization of IgG and complement (C_3) in the basement membrane zone of three typical cases of bullous pemphigoid and three atypical variant cases (nodular, polymorphic and localized pemphigoid, respectively). There was no significant difference in the *in vivo* ultrastructural localization of these immunoreactants between the typical and the atypical cases of pemphigoid. In all cases IgG and C_3 were detected between the basilar surface of the basal keratinocytes and the basal lamina. Moreover, the *in vitro* binding sites of circulating anti-basement membrane zone antibodies were identical in the case of nodular pemphigoid and a typical pemphigoid as control. We also observed that immunoreactants were not deposited beneath melanocytes.

The characteristic cutaneous lesion of bullous pemphigoid (BP) is a tense blister arising on apparently normal skin or on an erythematous base. Histopathology shows a sub-epidermal split. Routine immunofluorescence (IF) demonstrates the deposition of immunoreactants (IgG and C_3) in a linear pattern along the basement membrane zone (BMZ), usually with circulating anti-BMZ antibodies in the serum.[1] However, the same routine IF findings are shared by a number of clinical variants of BP and also by epidermolysis bullosa acquisita (EBA). The atypical variants include nodular, vesicular, polymorphic, localized, vegetans and erythrodermic forms of BP.[2–7] EBA is an acquired mechano-bullous disorder which usually presents as blistering, scarring and milia formation on the extremities. However, cases of EBA may initially masquerade as 'typical' BP and prove difficult to treat.[8] Ultrastructural studies have shown that in EBA, IgG is deposited in the lamina densa and sub-lamina densa region, whereas in BP the antibodies are localized in and above the lamina lucida.[9]

The purpose of the present study was to define by immunoelectron microscopy (IEM) the ultrastructural site of deposition of immunoreactants in three atypical cases of BP, in order to

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distinguish them from EBA. We also wanted to determine whether there were any differences in the ultrastructural localization of immunoreactants between these atypical cases and clinically typical BP, as in most previous reports this question has not been answered.[10]

METHODS

Patients

Three patients (Cases 1–3) were diagnosed as having variants of BP. They were compared with three patients with clinically typical BP as controls. In all cases the diagnosis of BP was confirmed by positive direct IF showing linear IgG and C_3 deposits in the BMZ of perilesional skin, and by the presence of circulating IgG class anti-BMZ antibodies (titre > 1:40) detected by indirect IF.

Case 1. Nodular BP. A 50-year-old woman had typical BP which went into remission on treatment. Three years later numerous prurigo nodularis-like lesions developed on the legs and trunk, and the titre of circulating anti-BMZ antibodies rose from 1:10 to 1:160.

Case 2. Polymorphic BP. A 72-year-old woman had a 6-month history of pruritic vesicles and erythema similar to dermatitis herpetiformis on the trunk and extremities.

Case 3. Localized BP. An 80-year-old woman had recurrent blister formation on a localized area of her back which resulted in a circumscribed plaque with pigmentation and erythema.

Direct immunoelectron microscopy

Perilesional skin biopsies were taken from all patients. To preserve the antigenicity and morphological detail the fresh, unfixed biopsy material was immediately immersed in phosphate-buffered saline (PBS) with 10% glycerol, pH 7·2 at 4°C for 90 min. After embedding in OCT compound the skin was stored at −70°C. Immediately before each experiment 6 μm cryostat sections of frozen skin were cut and incubated in a moist chamber at room temperature for 60 min with horseradish peroxidase labelled rabbit anti-human IgG (γ chain) anti-serum (1:20 dilution; Dakopatts Co.) or rabbit anti-human C_3 anti-serum (1:10 dilution; Dakopatts Co.). The sections were rinsed in PBS, fixed in 2% glutaraldehyde solution for 30 min at 4°C, rinsed again and preincubated in 3,3′-diaminobenzidine (DAB) solution for 30 min. Following incubation in DAB-hydrogen peroxide solution for 3 to 10 min and rinsing in PBS, the sections were postfixed with 1% osmium tetroxide for 30 min and dehydrated in a graded series of alcohols. They were then embedded in epoxy resin using the inverted gelatin capsule method. Ultrathin sections were observed under a transmission electron microscope (HU-12AS), both with and without counterstaining with uranyl acetate and lead citrate. Controls were prepared using normal rabbit serum (1:10 dilution) as the first reagent instead of the above antisera.

Indirect immunoelectron microscopy

Normal human skin, prepared as described above, was cut into 6 μm cryostat sections, as substrate for indirect IEM. Sections were incubated in 1:10 dilutions of serum from the patient with nodular pemphigoid (Case 1) (anti-BMZ antibody titre 1:160) and also in serum from a case of clinically typical pemphigoid with the same anti-BMZ antibody titre. After rinsing in PBS the sections were incubated with horseradish peroxidase labelled rabbit anti-human IgG antiserum (1:20 dilution). The sections were then treated as described above and the *in vitro* binding sites of IgG class anti-BMZ antibodies were observed.

TABLE 1. Immunoelectron microscopic localization of IgG and C_3 in the basement membrane zone of perilesional skin

	IgG	C_3
Nodular pemphigoid	BS	BS,LL
Polymorphic pemphigoid	BS,LL	BS,LL,BL
Localized pemphigoid	BS,LL	BS,LL
Controls: Typical bullous pemphigoid		
Case 1	BS,LL	BS,LL
Case 2	BS,LL	BS,LL
Case 3	BS,LL	BS,LL

BL: basal lamina
BS: basilar surface of the basal cells
LL: lamina lucida

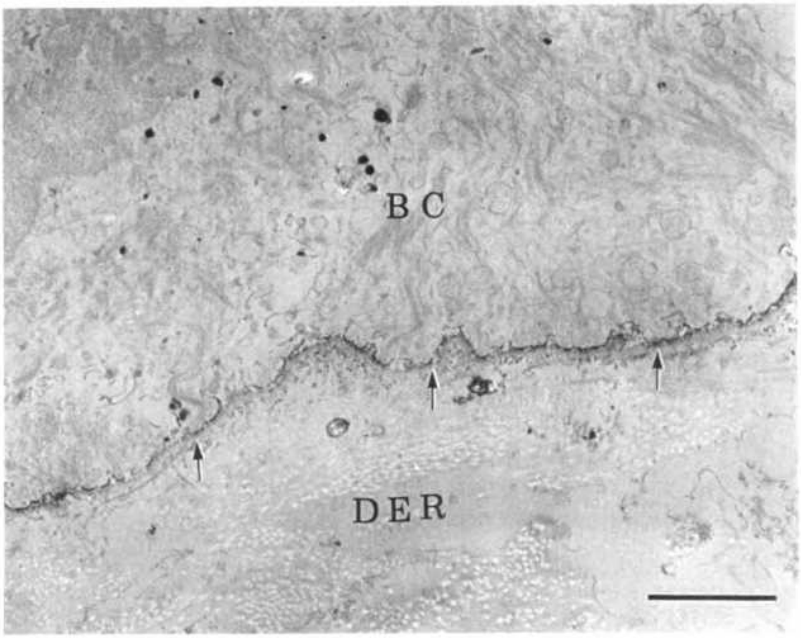

FIGURE 1. Direct immunoelectron micrograph of the basement membrane zone from the patient with localized pemphigoid. IgG is distributed in a linear pattern between the basilar surface of the basal cells and the basal lamina (arrows). No deposition of IgG was observed below the basal lamina or in the cytoplasm of the basal cells. Section counter-stained with uranyl acetate and lead citrate. BC = basal keratinocyte; DER = dermis; Bar = 1 μm.

RESULTS

Direct immunoelectron microscopy

The site of deposition of immunoreactants is shown in Table 1. We found no significant difference in the site of deposition of IgG or C_3 between the typical and the atypical cases of BP. In all cases IgG or C_3 or both were distributed linearly, but not homogeneously, between the basilar surface of the basal keratinocytes and the basal lamina. There was no evidence of *in vivo* deposition of immunoreactants either below the basal lamina or within the cytoplasm of the basal keratinocytes (Fig. 1). In all cases the reaction product was more pronounced on the basilar surface of the basal keratinocytes than in the lamina lucida. The case of nodular pemphigoid showed no IgG deposition in the lamina lucida. The findings in the case of polymorphic pemphigoid varied within the specimen. In some areas there was evidence of IgG deposition on the basal lamina, but at other sites in the same specimen, the reaction product was confined to the basilar surface of the basal keratinocytes closely related to the attachment plaque of the hemidesmosomes.

In none of the six cases was reaction product seen beneath melanocytes (Fig. 2).

Indirect immunoelectron microscopy

The *in vitro* binding sites of circulating anti-BMZ antibodies were the same in the patient with nodular pemphigoid and the control patient with typical pemphigoid. In both cases the reaction product was observed mainly around the intracytoplasmic hemidesmosomes and the basilar surface of the basal keratinocytes (Fig 3a and b), but not beneath the melanocytes.

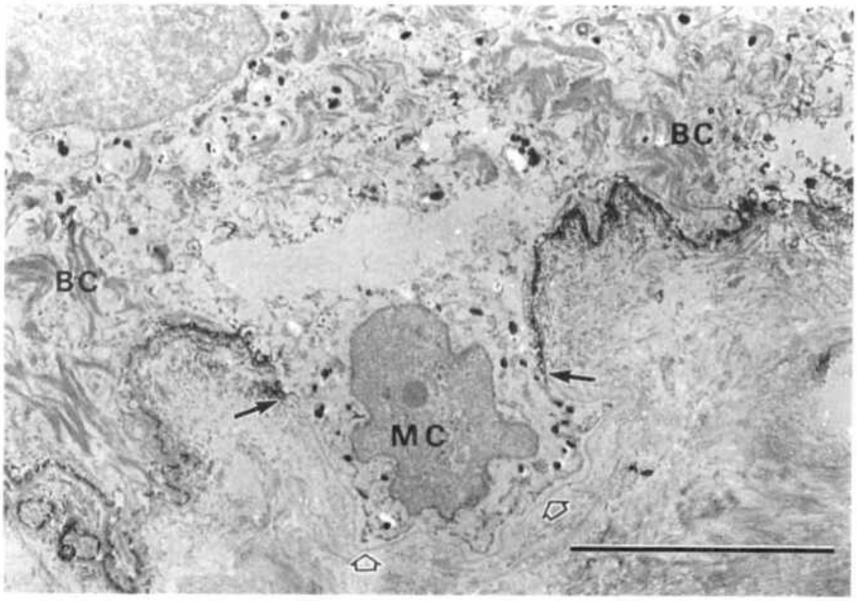

FIGURE 2. Direct immunoelectron micrograph of *in vivo* deposition of IgG in the basement membrane zone of the case of polymorphic pemphigoid. The reaction product is present beneath the basal keratinocytes (BC) but is absent beneath a melanocyte (MC). Solid arrows indicate the boundary between the basal cells and the melanocyte. Open arrows indicate the basal lamina. Section counter-stained with uranyl acetate and lead citrate. Bar = 5 μm.

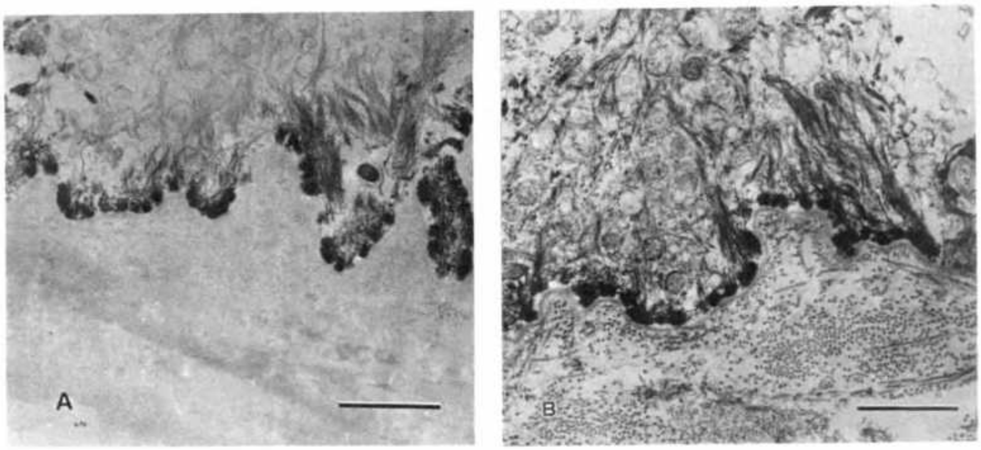

FIG. 3. Indirect immunoelectron micrographs of *in vivo* deposition of circulating anti-BMZ antibody in the patient with nodular pemphigoid (Case 1). Immunoreactant is mainly deposited with the cytoplasm of the basal cells, especially around hemidesmosomes. (A): unstained section (B): section counterstained with uranyl acetate and lead citrate. Note that the basal lamina can be clearly seen in B. Bars = 1 μm.

DISCUSSION

In this study we have found no difference in the ultrastructural binding sites of *in vivo* immunoreactants and of circulating anti-BMZ antibodies between three clinically atypical cases of pemphigoid and three control patients with 'classical' BP. None of the three atypical cases showed evidence of binding of immunoreactant below the basal lamina, thus confirming that these were indeed variants of BP and not EBA. This may be of more than academic interest as the latter condition may prove resistant to treatment[8]. Because of the small number of cases in the present study it is not possible to be certain that all the atypical forms of BP would have similar ultrastructural findings. Our findings suggest that the clinical appearance of BP lesions may be determined by factors other than the binding site of immunoreactants. For example, it is possible that clinical atypicality may be in some way related to heterogeneity of the BP antigen.[11-13]

Recently, the use of an antigen-mapping direct IF technique has made it possible to determine approximately the ultrastructural localization of anti-BMZ immunoreactants without the need for IEM.[14,15] The use of the sodium chloride split-skin technique for indirect IF would also demonstrate whether circulating antibodies are binding above or below the lamina.[16] However, neither of these techniques show the fine detail provided by IEM.

There is conflicting evidence regarding the presence of BP antigen in the BMZ beneath melanocytes.[17,18] Our observations support the concept that melanocytes do not express the BP antigen, and that the BP antigen is produced by basal keratinocytes in close association with hemidesmosomes and secreted into the lamina lucida.[19,20]

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Clinical markers of androgenicity in acne vulgaris

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SUMMARY

Androgenic stimulation of sebaceous glands is necessary for development of acne. If hyperandrogenaemia were a major determinant of acne in women, the frequency of other clinical markers of androgenicity should increase with acne severity. To investigate this, 268 female subjects (aged 12–44 years) were studied. Subjects were divided into groups on the basis of acne severity: physiological, moderate, and severe. With exclusion of women taking oral contraceptives or anti-androgen therapy, subjects in each group were similar with respect to age at menarche and incidence of menstrual irregularity or amenorrhoea. Reports of excessive body hair, and clinical hirsutes on examination were few and there were no significant differences between acne severity groups. No correlation was observed between acne and hirsutes grades in all subjects (rank correlation coefficient = 0·096). Mild male pattern androgenic alopecia occurred in similar proportions of subjects in the three groups. Female pattern androgenic alopecia was observed in only two subjects.

We have shown no correlation between acne severity and clinical markers of androgenicity in women. This suggests that in most cases factors other than hyperandrogenaemia are necessary for the development of acne.

The pathogenesis of acne vulgaris is multifactorial and our current understanding of it is incomplete. One major factor appears to be an increased rate of sebum excretion which correlates with acne severity.[1] Sebum excretion is principally under hormonal control with androgens providing the major stimulus.[2] The importance of the relationship between androgens and acne is illustrated by the clinical improvements achieved by anti-androgen therapy in women with acne.[3]

A number of studies have documented evidence of raised androgen levels in groups of women with acne.[4–11] Elevation of both total and free (unbound) circulating androgens have been found. Evidence from other studies suggests that sebaceous glands in subjects with acne may be

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more sensitive than those in normal subjects to the effects of normal levels of circulating androgens.[12–14]

The extent to which circulating androgens and additional factors operate in acne is as yet unknown. It has been our observation that most patients attending dermatology clinics with acne do not have other features of androgenicity such as hirsutism, menstrual irregularity and amenorrhoea. We therefore undertook a study to examine the relationship between acne severity and clinical markers of hyperandrogenism.

METHODS

The study comprised 268 females (age range 12–44 years). Of these, 218 were drawn consecutively from patients attending the dermatology department presenting with acne vulgaris, who satisfied inclusion criteria and who consented to inclusion in the study. Fifty subjects with mild or no acne were also studied to provide greater numbers at the lower range of acne severity. These subjects consisted of volunteer members of the hospital staff. Only Caucasian females were included to minimize racial variations, particularly in hirsutism. Subjects were blind to the reasons for the study.

Each subject was questioned with regard to menstrual history. Established definitions of menstrual irregularity and secondary amenorrhoea do not exist. After consultation with colleagues at the Leeds University department of Gynaecology, we chose arbitrarily to define normal menstruation as a usual menstrual cycle length of 28 ± 7 days. Irregular cycles were defined as cycle length <21 days or >35 days, or cessation of menstruation for more than 3 months within the previous 2 years. Amenorrhoea was defined as cessation of menstruation for 3 months or more at the time of study. Subjects were also asked about excessive body hair. Assessment of fertility was considered inappropriate in subjects of this age group.

Subjects were examined by two observers (R.S.-D. and B.H.). In order to minimize bias, the acne patients were seen whilst receiving effective acne therapy. For these patients, the two observers were unaware of the acne grade before treatment, which had been assigned by a different observer (W.J.C). For the other volunteer subjects it was necessary to assign an acne grade at the time of inclusion in the study. Acne severity was graded on a scale of 0–10 for the face, chest and back using the method of Burke and Cunliffe,[15] the sum of grades at the three sites providing a total grade. Hirsutes was graded by the method of Ferriman and Gallwey.[16] Hirsutes grades in excess of 6 were considered indicative of clinical hirsutism. Female pattern androgenic alopecia was graded on a scale of 0–3 as defined by Ludwig.[17] Male pattern androgenic alopecia was graded on a scale of 1–8 as defined by Hamilton.[18] Keratosis pilaris, acanthosis nigricans and hidradenitis suppuritiva have been associated with androgenicity.[19,20] The presence and extent of these conditions was noted.

Because acne occurs to some extent in most of the population from adolescence up to the fourth decade,[21] a control group consisting entirely of subjects without acne was not used. Subjects without acne or with physiological acne (grade $<0{\cdot}5$ at each site; total grade $<1{\cdot}0$) were compared with patients with moderate acne (grade $>0{\cdot}5$, $<1{\cdot}5$ at each site; total grade $>1{\cdot}0$ and $<3{\cdot}0$) and severe acne (grade $>1{\cdot}5$ at each site; total grade $>3{\cdot}0$).

Subjects who had received oral contraceptives within 3 months of the study, or other drugs likely to interfere with androgen metabolism within 6 months of the study (e.g. cimetidine, spironolactone, or cyproterone acetate), were considered separately from the other subjects, as these agents may have had effects on clinical markers of androgenicity.

Statistical analysis comparing acne and hirsutes grades was performed using Spearman's rank correlation coefficient, as neither acne nor hirsutes grades were normally distributed. Analyses of data comparing physiological or no acne, moderate and severe acne utilized one-way analysis of variance and Chi square tests where indicated.

RESULTS

One hundred and ninety subjects had received no hormonal therapy; 77 were in the physiological acne range (Group 1), and of these five had no acne. Sixty eight subjects had moderate acne (Group 2). Forty five subjects had severe acne (Group 3); the most severe total acne grade was 14·0. The ages of the subjects were not significantly different in the three groups. Details of these three groups are given in Table 1.

There was no significant difference in mean age at menarche between the three groups. The differences in incidence of menstrual irregularity and amenorrhoea between the three groups were not significant (Fig. 1).

The proportion of subjects reporting excessive body hair in the three groups was similar. The incidence of clinical hirsutes on examination (grade > 6) was low in all three groups and there was no significant difference between them. The relationship between acne and hirsutes grades is shown in Fig. 2. There was no significant correlation between acne and hirsutes grades, with a Spearman rank correlation coefficient of 0·096.

TABLE 1. Clinical characteristics of subjects studied, excluding patients on hormonal therapy

	Group 1 (physiological or no acne)	Group 2 (moderate acne)	Group 3 (severe acne)	*P*
No. of subjects	77	68	45	—
Age (years) (mean and range)	22·8 (12–44)	22·0 (13–41)	22·0 (13–42)	NS
Age at menarche (years) (mean and range)	12·6 (10–18)	12·8 (11–17)	12·3 (10–15)	NS
Menstrual dysfunction				
Irregular cycles	9 (11·7%)	12 (17·6%)	7 (15·6%)	NS
Amenorrhoea	2 (2·6%)	1 (1·5%)	2 (4·4%)	NS
Excessive body hair	13 (16·9%)	10 (14·7%)	4 (8·9%)	NS
Clinical hirsutes	5 (6·5%)	4 (5·9%)	1 (2·2%)	NS
Male pattern alopecia				
Grade 1	69 (89·6%)	55 (80·9%)	35 (77·8%)	NS
Grade 2a	8 (10·4%)	13 (19·1%)	10 (22·2%)	NS
Female pattern alopecia				
Grade 0	77 (100%)	66 (97·1%)	45 (100%)	—
Grade 1	— —	2 (2·9%)	— —	—
Keratosis pilaris	5 (6·5%)	6 (8·8%)	3 (6·7%)	NS
Hidradenitis suppuritiva	— —	1 (1·5%)	— —	—
Acanthosis nigricans	1 (1·3%)	— —	— —	

NS = Not significant

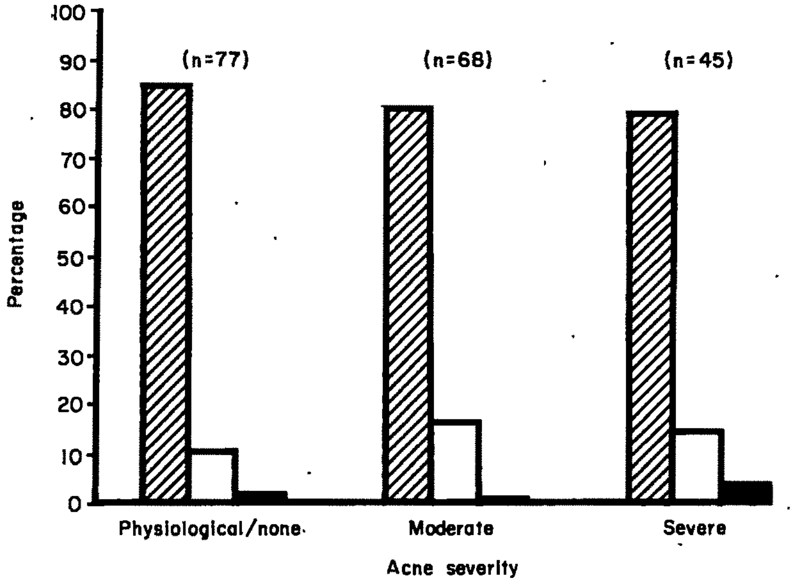

FIGURE 1. Incidence of menstrual irregularity and amenorrhoea in subjects not receiving hormonal therapy. ▨ regular cycles; ☐ irregular cycles; ■ amenorrhoea.

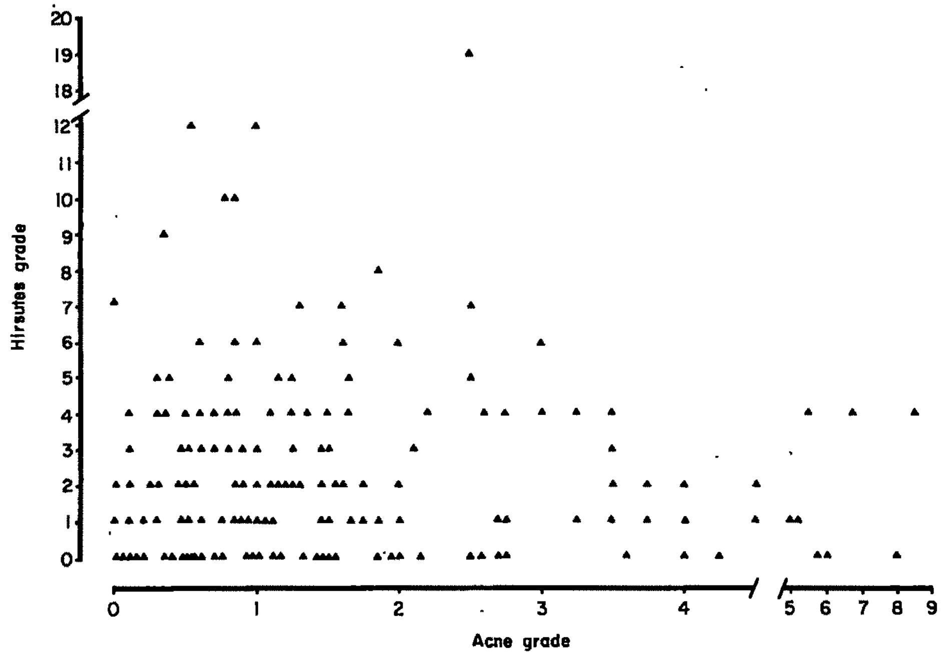

FIGURE 2. Relationship between hirsutes grade and acne grade in subjects not receiving hormonal therapy.

TABLE 2. Details of subjects receiving hormonal therapy

	Physiological/no acne	Moderate acne	Severe acne	*P*
No. of subjects	26	24	28	
Hormonal therapy				
Oral contraceptives				
Low oestrogen (< 35 μg)	20 (76·9%)	14 (58·3%)	18 (64·3%)	—
High oestrogen (> 35 μg)	1 (3·8%)	— —	1 (3·6%)	—
Progesterone only	1 (3·8%)	— —	— —	—
Unknown	2 (8·3%)	3 (12·5%)	2 (7·1%)	—
Cyproterone acetate/oestrogen (Diane)	2 (8·3%)	6 (25·0%)	6 (21·4%)	—
Spironolactone	— —	1 (4·2%)	1 (3·6%)	—
Age years/(mean and range)	21·3 (17–32)	22·7 (16–27)	24·2 (16–37)	NS
Age at years menarche (mean and range)	12·7 (10–15)	13·2 (11–16)	12·6 (11–16)	NS
Menstrual dysfunction				
Irregular cycles	6 (23·1%)	5 (20·8%)	3 (10·7%)	NS
Amenorrhoea	— —	1 (4·2%)	— —	—
Excessive body hair	2 (8·3%)	4 (16·7%)	2 (7·1%)	NS

Two subjects in Group 2 (2·9%) had grade 1 female pattern androgenic alopecia (mild balding over the vertex and frontal scalp). No subject in groups 1 or 3 had evidence of female pattern androgenic alopecia. No subject had greater than grade 1 alopecia. Male pattern androgenic alopecia grade 2a (mild bitemporal recession) was observed in similar proportions of patients in groups 1, 2 and 3. No subject had greater than grade 2a alopecia.

Keratosis pilaris was present in a small proportion of each of the three groups. The differences between the groups were not statistically significant. Hidradenitis suppuritiva was present in a mild form confined to the axillae in 1 subject in Group 2 but in none of the subjects in Groups 1 and 3. Acanthosis nigricans was observed in 1 subject in Group 1, but in none of the subjects in Groups 2 and 3.

A total of 78 subjects had received hormonal therapy. Sixty two had taken oral contraceptives (in all cases solely for contraceptive purposes), and 16 anti-androgen therapy (in all cases solely for acne). Of these, 26 had physiological, 24 moderate, and 28 severe acne. Details of this group are shown in Table 2. In these subjects the mean age at menarche was similar to that of the subjects not on hormonal therapy. In addition, menstrual irregularity and excessive body hair prior to hormonal therapy were reported in a similar frequency to that in subjects not on hormonal therapy.

DISCUSSION

Acne vulgaris is an accepted feature of hyperandrogenism in women with polycystic ovary syndrome, adrenal hyperplasia, and androgen secreting tumours.[22] Other features in these patients which suggest a hyperandrogenic state include menstrual irregularity, hirsutism, androgenic alopecia, and according to some authors, hidradenitis suppuritiva, acanthosis nigricans and keratosis pilaris.[18,19]

Several observations implicate androgens in the pathogenesis of acne. Acne severity correlates with sebum excretion rate and androgens are the principal hormonal agents controlling this process.[2,1] Also, anti-androgen therapy improves acne in women.[3] Testosterone and dehydroepiandrosterone sulphate (DHA-S) are generally considered to be the most important circulating androgens in women, and it is the free non-protein bound fractions which have the greatest biological activity. In the case of DHA-S, conversion to the more biologically active 17β-hydroxysteroids is probably responsible for most of its androgenic activity.[23] A number of studies have demonstrated raised levels of circulating androgens, and in particular free testosterone in women with acne, mainly in selected severe or therapy resistant cases.[4–11] However, significant numbers of women with raised androgen levels have no acne[8] and women with acne may have normal androgen levels.[7,24] It is likely that there is individual variation in the response of sebaceous glands to circulating androgens. One possible mechanism is individual variation in conversion of circulating androgens to more active metabolites in the skin. Recent studies have provided evidence that conversion of testosterone to the more active dihydrotestosterone by the enzyme 5-alpha reductase is increased in acne subjects.[12–14] Differences in activity of this enzyme at different skin sites might also explain the frequent clinical observation of patients with severe acne at one acne site and no acne at another.

If raised circulating androgen levels were a major factor in the development of acne, some degree of correlation might be expected between acne severity and the incidence of other clinical manifestations of hyperandrogenaemia.

Three previous studies have addressed the problem of acne and hyperandrogenism. Steinberger *et al.*[5] found an association between acne, hirsutes and menstrual disturbance. However, their subjects were selected in that they were resistant to acne therapy. Held *et al*[25] studied a group of women attending a dermatology department and compared those presenting with acne and patients with other complaints. Again an association was found between acne and hirsutism and an association with menstrual irregularity just reached significance. Their definition of hirsutism was merely of coarse hairs in abnormal sites and the racial background of the patients was not stated.

Reingold and Rosenfield[24] studied the relationship between mild hitsutism or acne and androgen levels in women and although acne and hirsutism were found to be associated with a significantly raised testosterone level, the most important finding was the striking variability in the relationship between acne or hirsutism and free testosterone levels. In our study no correlation was found between acne and hirsutes, nor a relationship of acne with menstrual irregularities. In addition we were unable to show any increase in the incidence of male and female pattern androgenic alopecia with increasing acne severity. The incidence of keratosis pilaris, hidradenitis suppuritiva and acanthosis nigricans was low at all acne grades. Our data is consistent with the findings of Reingold and Rosenfield[24] in that a small subset of patients with acne showed cutaneous features of hyperandrogenism, but this was independant of the severity of acne.

A number of factors may have introduced bias into our results. Refusal of subjects to be included in the study may have introduced bias if hyperandrogenism had been more frequent in those who refused. The incidence of hyperandrogenism in this group cannot be determined, however the numbers who refused to be interviewed were relatively small (2%). For the majority of subjects, the observers were unaware of the initial acne grade. However, in a small proportion of subjects (mostly with lower acne grades), the observers were aware of the acne grade and this may have introduced bias. The use of physical methods for removal of hair by subjects with hirsutes may have affected hirsutes grades at the time of examination. However, the proportions of subjects aware of excess body hair and thus likely to employ these methods were similar for the three acne severity groups.

Subjects receiving hormonal therapy may have had hyperandrogenism (in particular hirsutism) which was masked by these treatments, and this may have introduced bias. However, no subject had received hormonal therapy wholly or partly for hirsutism and the proportion of these subjects reporting excessive body hair was similar to that of subjects not on hormonal therapy for all grades of acne severity. In addition, separate analysis of subjects taking hormonal therapy revealed similarities to subjects not on hormonal therapy with respect to age at menarche and incidence of menstrual irregularity prior to hormonal therapy.

It is clear that androgenic stimulation of sebaceous gland activity is a pre-requisite for the development of acne. In addition, acne can be a feature of hyperandrogenic states. In this study we have demonstrated no correlation between acne severity and clinical markers of androgenicity, and have found that the majority of women with acne show no abnormality in other androgen target organs. These observations suggest that the increased sebaceous gland activity which occurs in acne is in most cases not a result of hyperandrogenaemia. Other factors appear to be operating, and an abnormal response of the pilosebaceous unit to normal circulating androgens seems to be the most likely explanation.

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Papillon-Lefèvre syndrome: a study of the long-term clinical course of recurrent pyogenic infections and the effects of etretinate treatment

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SUMMARY

A family with a clinical variant of Papillon-Lefèvre syndrome (PLS), associated with recurrent pyogenic infections, has been followed up for more than 20 years. Of the five living siblings, four were treated with etretinate for a period of at least 21 consecutive months. Clinical follow-up showed that the course of recurrent infections in susceptible PLS patients, although usually more severe in childhood, can be variable and unpredictable. The etretinate therapy resulted in marked improvement of the keratodermas, and was associated with complete remission of the pyodermas on both keratotic and non-keratotic skin. It is, therefore, suggested that etretinate may have a primary role in the prevention of recurrent pyogenic infections in susceptible PLS patients.

Papillon-Lefèvre syndrome (PLS) is a hereditary condition characterized by diffuse transgredient palmoplantar keratosis and premature loss of both the deciduous and permanent teeth. An increased susceptibility to infections, which has been observed in about 20% of PLS patients, is considered now to be a possible symptom in this syndrome[1] (E. Haneke, personal communication 1986).

Etretinate has been shown recently to be an effective treatment for the skin lesions of patients with PLS.[2–7] There is still, however, a relative lack of clinical data regarding the long-term course of recurrent infections in susceptible PLS patients and the effects of etretinate treatment on these.

In 1965, Haim and Munk[8] described a Jewish family from Kerala, South India, in which six out of eight siblings were affected by a syndrome which was later recognized as a clinical variant

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of PLS.[1,9,10] In addition to the characteristic transgredient keratosis palmaris et plantaris, hyperkeratotic patches over the elbows and knees, and periodontosis which led to the loss of the permanent teeth at an early age, these patients had arachnodactyly, with a peculiar deformity of the terminal phalanges and an increased susceptibility to recurrent pyogenic infections of the skin and internal organs.[8]

The family described by Haim and Munk[8] has been observed and followed up for more than 20 years. Of the five living siblings, four were treated with etretinate for a period of at least 21 consecutive months. We describe here the long-term clinical course of recurrent pyogenic infections in these PLS patients, and the effects of etretinate treatment on that course.

METHODS

The patients' clinical files were reviewed and each of the five living patients (two males and three females) aged 21–40 years, was interviewed and examined. Specific attention was given to the clinical course of recurrent infections.

At the time of examination, one patient (Patient 3) had already been receiving 0·5 mg/kg/day etretinate orally, for 2 years. Three of the remaining four patients had severe palmoplantar hyperkeratosis and large keratotic plaques over the elbows and knees, with severe functional limitation and social embarassment. This prompted us to initiate treatment with etretrinate. Each patient received an initial oral daily dose of 1 mg/kg etretinate. With maximal clinical improvement, the dosage was reduced to a maintenance level of 0·4–0·5 mg/kg/day. Treatment was continued, and the patients had been followed up for 21 months at the time of writing. Assessment of their general condition, skin findings and monitoring of fasting serum triglycerides, cholesterol and liver enzymes, were performed at monthly intervals during the initial 6 months, and every 3 months thereafter.

Specific attention was given to the clinical course of recurrent infections while on etretinate therapy.

RESULTS

Clinical data on the six PLS patients (Table 1) shows that they had all suffered from recurrent pyogenic infections during at least one period of their lives. In infancy and childhood these infections were severe as they tended to involve internal organs, causing death in one patient (Patient 1) and a liver abscess with perforation into the lung in another (Patient 3). In adolescence, the infections became milder, as they were confined to the skin. In adulthood, prior to etretinate treatment, spontaneous remission of the pyodermas occurred in two patients (Patients 2 and 5), no change in the course of the pyodermas was recorded in two patients (Patients 3 and 4), and another patient (Patient 6) began to suffer from recurrent infections only at the age of 20 years. In all cases bacteriological cultures yielded *Staphylococcus aureus*.

It should also be noted that tuberculoid leprosy was diagnosed in Patient 2 at the age of 17 years. This was most probably acquired in India, her former country of residence. She was, therefore, treated at a leprosy clinic with dapsone for several years until all signs of active disease had disappeared.

In all the patients newly treated with etretinate (Patients 2, 4 and 6) maximal resolution of the skin lesions was recorded 4–8 weeks after the initiation of therapy. Some worsening of the skin lesions was noted upon lowering the dosage to the maintenance level. However, there was still a significant improvement of the keratoderma, absence of fissures (Figs 1 and 2), painfree palms and soles, and improvement in gait and manual mobility.

TABLE 1. The clinical course of infections and the effects of etretinate treatment in the six siblings affected by Papillon-Lefèvre syndrome

Patient no.	Age at time of writing (years)	Sex	Infancy and childhood (< 12 years)	Adolescence (12–20 years)	Adulthood (> 20 years)	Etretinate treatment	
						Duration	Effects
1	—	M	Died at 12 years from abdominal abscess	—	—	—	—
2	42	F	Recurrent *S. aureus* abscesses of skin	No change in course of pyoderma; tuberculoid leprosy	Since age 28, completely free of pyoderma; no sign of active leprosy	21 months	Marked improvement of keratoderma
3	38	M	Liver abscess with perforation into lung	Recurrent *S. aureus* abscesses, limited to axillae and lower limbs	No change in course of pyoderma	45 months	Marked improvement of keratoderma and complete remission of pyoderma
4	34	F	Deep *S. aureus* abscesses in soft tissues of right leg	Recurrent *S. aureus* abscesses, limited to affected hyperkeratotic skin	No change in course of pyoderma	21 months	Marked improvement of keratoderma and complete remission of pyoderma
5	31	M	Recurrent abscesses, mainly on affected hyperkeratotic skin	No change in course of pyoderma	Since age 20, constant and gradual diminution in severity and frequency of pyoderma; recently in complete remission	—	—
6	23	F	—	—	Since age 20, recurrent *S. aureus* pyoderma in affected and non-affected skin	21 months	Marked improvement of keratoderma and complete remission of pyoderma

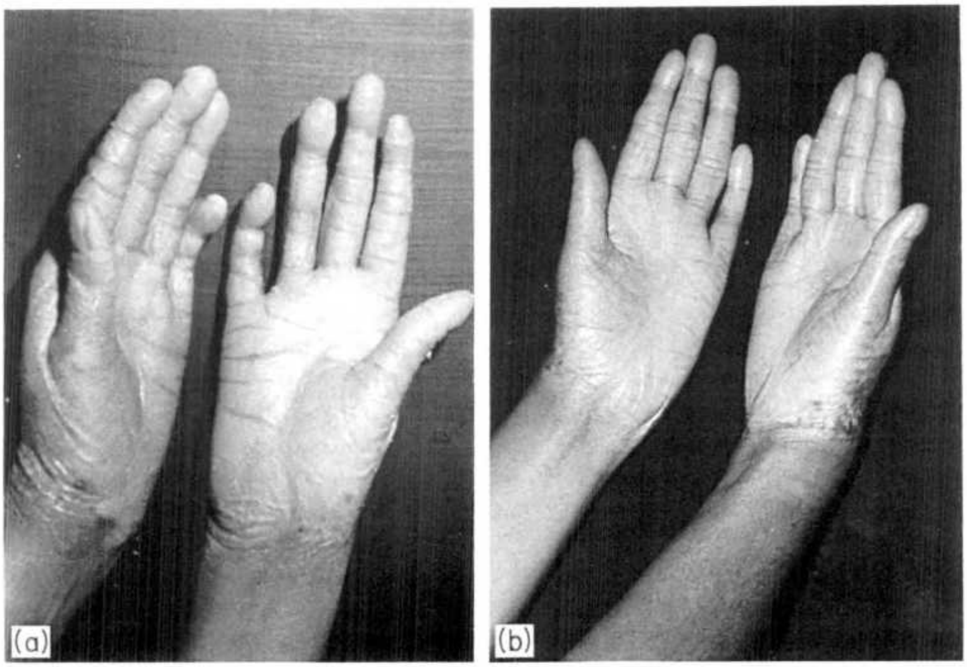

FIGURE 1. Patient 3. (a) Hyperkeratosis of the palms with fissures. Note the deformity of the terminal phalanges. (b) Appearance of palms after 4 months treatment with etretinate 0·5mg/kg/day. Note the clearing of the keratoderma.

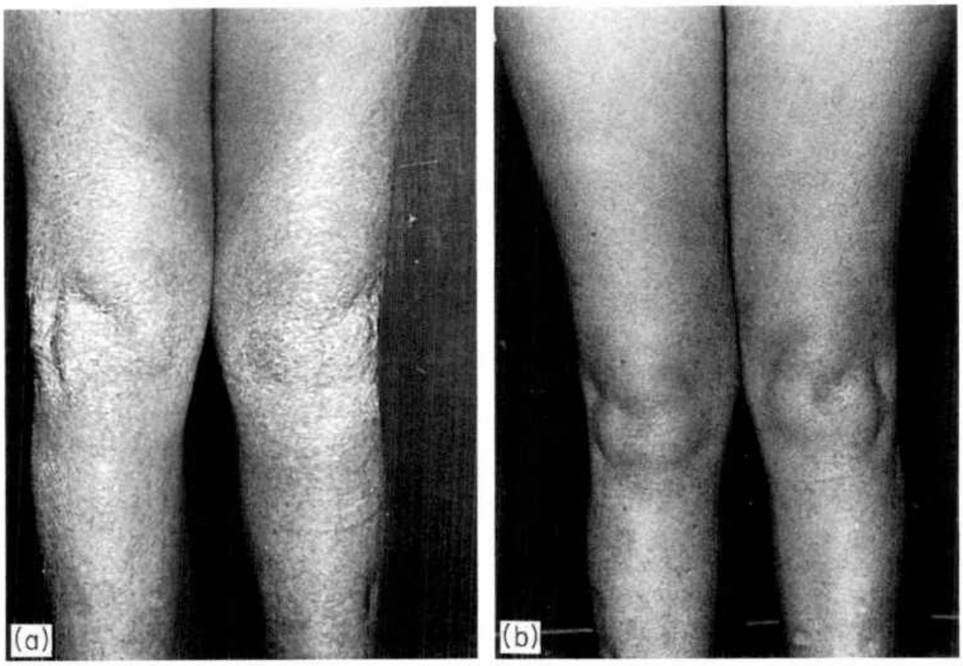

FIGURE 2. Patient 3(a) Hyperkeratotic patches over the knees (b) Appearance of knees after 4 months treatment with etretinate 0·5mg/kg/day. Note clearing of the hyperkeratosis.

In addition, the two patients (patients 4 and 6) who were still affected by recurrent pyoderma at the time of initiation of treatment obtained complete remission of the infection during the entire course of etretinate therapy. Patient 3, who began etretinate treatment 2 years before his siblings, had been free of pyoderma ever since (Table 1).

Despite the usual side effects of etretinate therapy which were relatively mild, all patients were satisfied with the results of their treatment.

DISCUSSION

The skin is reported to be the most common site affected by infections in susceptible PLS patients. Internal organs, such as the liver, lung, kidney and abdominal cavity are less frequently involved[1,5] (E. Haneke, personal communication, 1986). In our patients the pyogenic infections tended to be internal, severe and in one patient fatal at an early age. In adolescence, these infections became milder as they were confined to the skin. In adulthood the clinical course was more variable, as spontaneous remission occurred in two siblings, no change was observed in two and worsening was recorded in another.

Bravo-Piris *et al.*[5] described a patient with PLS who did not show any improvement in his recurrent infections with time. This patient subsequently died of massive pneumonia in early adulthood. Thus, it appears that in the individual patient with PLS, susceptibility to infections can have a variable and unpredictable course.

The underlying mechanism for susceptibility to infections in PLS patients remains to be determined. A high prevalence of polymorphonuclear leukocyte disorders and deficiencies in host defence mechanisms has been observed in patients with precocious peridontal disease.[11,12] These observations and the susceptibility of some PLS patients to recurrent infections have prompted investigations of the immune status of PLS patients. The results of these studies, however, have been inconclusive. Some investigations have shown disturbances in both polymorphonuclear leukocyte motility and bactericidal functions[5,12–14] or in bactericidal functions alone,[15,16] whereas others did not demonstrate any defects at all.[11,17] Similarly, disturbances in various lymphocyte stimulatory responses of PLS patients were observed in some studies,[18–20] but not in others.[11,15]

Etretinate has been shown to be an effective treatment for the keratoderma in PLS patients.[2–7] The marked improvement in keratoderma in our patients lends further support to these reports. The cessation of recurrent pyogenic infections during the entire course of etretinate therapy may point to a causal relationship. This may be due in part to the improvement in the keratotic lesions and diminution of fissuring in the palmoplantar skin. However, complete remission of the pyoderma occurred also in the two patients (Patients 3 and 6) who had suffered also from recurrent infections on clinically normal skin. Since none of the patients had received prophylactic antibiotics to prevent recurrent infections, nor had they changed their personal hygiene, it is suggested that etretinate may have had a direct role in the prevention of recurrent infections in these patients.

Retinoids have a diversity of effects on virtually all the components of immune function which have been studied to date.[21] Of special interest are the recent studies which show that retinoids in low concentrations stimulate the production and release of superoxide by polymorphonuclear leukocytes.[22,23] Thus, one suggested mechanism by which etretinate may reduce susceptibility to recurrent pyogenic infections in susceptible PLS patients is by correcting a possible defect in the bactericidal activity of their polymorphonuclear leukocytes. This possibility requires further investigation.

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Active vitamin D3 does not suppress recruitment of G_0 cells following injury

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SUMMARY

The effect of topically applied active vitamin D3 on epidermal regeneration was studied in eight healthy volunteers following sellotape stripping.

The mobilization of resting (G_0) cells was assessed by means of DNA flow cytometry, using a narrow window in the mid-S phase to monitor the progress of the recruited cells. Although vitamin D3 was applied at a concentration exceeding the range reported to be effective in psoriasis, we were unable to find any effect of active vitamin D3 on G_0 mobilization *in vivo*.

Topical application of active vitamin D3, either as 1α,25-dihydroxycholecalciferol (1,25-DCC) or as 1α,24-dihydroxycholecalciferol, with or without occlusion, has been reported to be effective in the treatment of psoriasis.[1–3] Reports that these substances inhibit the proliferation of keratinocytes both in culture[4] and in an animal model *in vivo*[5] strongly suggest that the antipsoriatic effects may be mediated by the normalization of epidermal cell cycle kinetics.

It has recently been established that an epidermal injury, such as sellotape stripping, causes the recruitment of resting (G_0) keratinocytes, and hence provides a useful model for the hyperproliferative aspects of psoriasis.[6] In the present study we have used this model to establish whether or not 1,25-DCC suppresses G_0 mobilization in human skin.

METHODS

Subjects

Eight paid volunteers (four males and four females, aged between 20 and 28 years) with no history of skin disease, took part in the study. Clinical and laboratory examinations were carried out at the start of the study to confirm that the subjects were in good health and laboratory

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examinations were carried out at the end of the study to exclude any intercurrent laboratory abnormalities.

Experimental procedure

In this randomized double-blind study, 1,25-DCC (Hoffmann-La Roche & Co. Basel, Switzerland) at a concentration of 2 μg/ml in medium chain triglyceride, or medium chain glyceride base only, was applied twice daily for 1 week to six sites on the back of each subject. Each site measured approximately 2 cm^2. About 0·2 ml of the solution was spread over each of the six sites with the stem of a pipette and allowed to dry for about 5 min. On day 9, after a 24 h rest period, the sites were stripped with adhesive tape until the wet, glossy stratum granulosum appeared (usually after 35–45 applications of tape). Treatment was resumed immediately thereafter and continued at 12 h intervals up to the time at which the site was biopsied. Biopsies were taken from each site 26, 40, 44, 48, 52 and 56 h after stripping and processed for examination by DNA flow cytometry. The procedure was repeated one month later in each subject with the alternative test solution. Medical Ethical Committee approval was obtained prior to the experiments.

Sample preparation and DNA staining

The biopsies were taken with a razor blade in conjunction with a metal guard[7] and immediately transferred to phosphate-buffered saline (without Ca and Mg, pH 7·2–7·4, Seromed L-1825). Samples measured approximately 6 mm^2 with an intradermal depth of 0·3 mm.

Biopsies were processed for cell cycle kinetic studies as described previously.[7] Briefly, the skin specimen was incubated in trypsin, DNA stained with propidium iodide and filtered for flow cytometry.

Flow cytometry

Cell cycle analysis was performed using a flow cytometer 50H (Ortho Instruments, Westwood, MA, U.S.A.) equipped with a 5W argon ion laser (164-05, Spectra Physics, Mountain View, CA, U.S.A.). The PI fluorochrome was excited at the 488 nm laser line and the fluorescence was measured with a high-pass filter RG 630 (Melles Griot, Zevenaar, The Netherlands). At least 50,000 cells were counted, at 200–500 cells/s. The data were stored and analysed with a PDP11/34 computer (Digital Equipment, Galway, Ireland). Both the area and the peak value of each fluorescence signal were measured.

Analysis of the changes brought about by tape stripping was based on the apparent changes in cell numbers at different points in the cell cycle.[6] Briefly, a 'window' was selected in the histogram. This was set relative to the position of the peak of diploid cells as shown in Figure 1. The occupation of this window could be determined at the different times after stripping and data could be plotted to determine the time of maximal occupation, the height of maximal occupation and the total size of the recruited cohort of cells.

The total size of the cohort of recruited cells could be determined in two different ways. First, by means of integration of the data shown in Figures 2 and 3. The integration was performed using the following formula[6]:

$$N_{\mathrm{cohort}} = \frac{\delta T^{\star} v}{\delta W} \, \mathrm{summ}_i(N_i - N_{\mathrm{norm}})$$

where

δT = sample interval time,

v = velocity of the recruited cells passing through the window,
δW = width of the window
N_i = percentage of cells in the window at time t_i.
N_{norm} = control percentage of cells in the window at time t_i.

Secondly, the minimal percentages of recruited cells could be determined by measuring the percentages of cells in S and G2 phases together in the individual histograms at the time of maximal window occupation. From that the means ± SEM could be determined. This results in an under-estimate, the real percentages being much higher.

RESULTS

No clinical side-effects and no laboratory abnormalities were observed during the study. In particular, no abnormalities in serum calcium levels were found.

The mean percentages of cells (± SEM) in window 1 (mid-S phase) at the different time points are given in Figures 2 and 3. Figure 2 shows results after treatment with 1,25-DCC and Figure 3 results after treatment with the base only.

Using the above formula, and given that $\delta T = 4$ h, v = 1/duration of S phase = 1/8 h, and $\delta W = 0{\cdot}2$, we calculated that the mean percentages of the total cohort of recruited cells (mean and SEM) were 44 ± 7·5% for the 1,25-DCC treatment and 39 ± 7% for the base treatment. We determined the mean minimal percentages of recruited cells (± SEM) as 22 ± 2% for the 1,25-DCC treatment and 24 ± 2% for the base treatment.

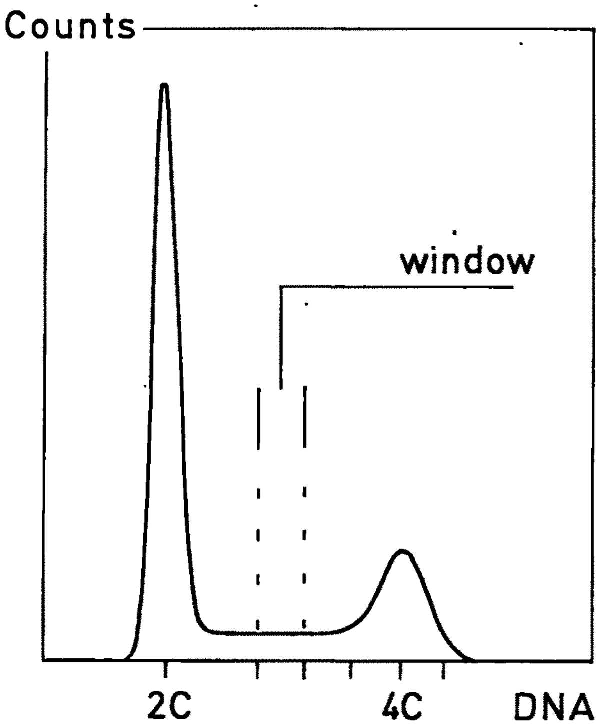

FIGURE 1. The distribution of diploid and tetraploid cells on flow cytometry. The window covers the maximum number of cells in mid-S phase.

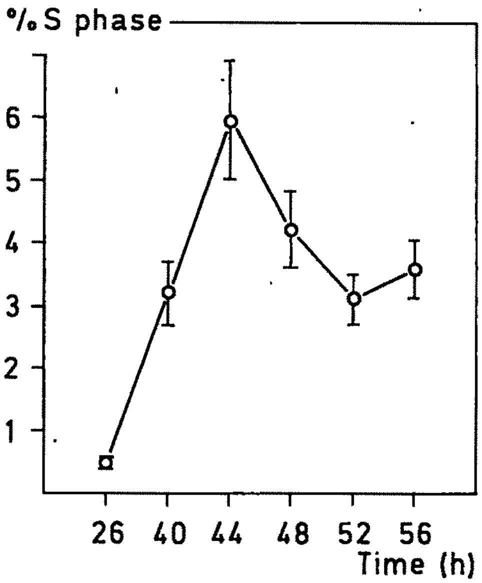

FIGURE 2. Percentages of cells in S phase at different times following Sellotape stripping of skin pretreated with 1,25-DCC. Points are mean ± SEM ($n=6$).

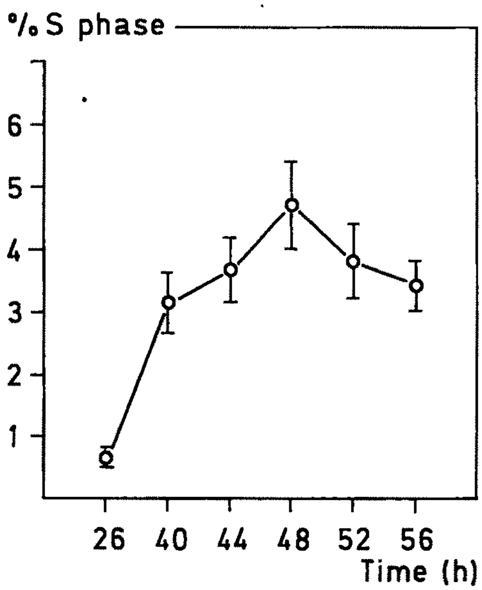

FIGURE 3. Percentages of cells in S phase at different times following Sellotape stripping of skin pretreated with the base only. Points are mean ± SEM ($n=6$).

As can be seen from Figures 2 and 3, 1,25-DCC does not delay but tends to accelerate the growth of the cohort of cells through the cell cycle. However, no significant changes could be shown in the time of maximal occupation, nor in the height of the cohort of cells (ANOVA; $P > 0{\cdot}05$).

DISCUSSION

The present investigation clearly shows that there is no significant inhibition of trauma-induced epidermal regeneration of human skin by 1,25-DCC. There was no change in time of maximal occupation, in height of the peak of maximal occupation or in the total size of the cohort of recruited cells. This observation is surpising in view of the successful treatment with active vitamin D_3, reported by several investigators.[1-3]

One explanation for the discrepancy between our observation and the successful treatment of psoriasis with 1,25-DCC could be suboptimal bioavailability of the drug in our study. However, in the present study, 1,25-DCC was applied twice daily, whereas the substance was applied once daily in a successful clinical study.[1] The concentration of 1,25-DCC used in the present study is at least four times higher than the concentration range used in the clinical trials,[1,3] the latter being in the range of 0·1–0·5 μg/g petrolatum. In the present investigation we did not use occlusion to enhance penetration of 1,25-DCC. However, one of the clinical studies claimed excellent results without occlusion.[2] Drug penetration through intact stratum corneum is lower than that through the parakeratotic horny layer of the psoriatic lesion. However, during successful treatment, the skin barrier recovers relatively early. As 1,25-DCC was also applied immediately after Sellotape stripping, from 26 to 56 h, good penetration, probably even better than that through a psoriatic lesion, was possible. Therefore, decreased bioavailability is very unlikely to be the explanation for the discrepancy between the clinical efficacy of 1,25-DCC and its lack of effect in the Sellotape stripping model.

The discrepancy between our findings and the reported antipsoriatic effectivity of 1,25-DCC might be explained by the possibility that tape striping of normal skin is not a good model for testing the effects of drugs which exert their clinical effects mainly in stable plaque psoriasis. Different 1,25-DCC receptor binding or interference by growth factors might be of relevance in this respect. In the suprabasal layers of the epidermis of the psoriatic lesion a two-fold increase of the binding capacity for epidermal growth factors has been observed compared with normal skin.[8] It is not known whether the cytosol binding of 1,25-DCC in lesional skin is comparable with that in normal skin. The binding capacity for growth factors and 1,25-DCC following Sellotape stripping are completely unknown. It has been shown in cultures of fibroblasts that 1,25-DCC only modulates growth of cells exposed to growth factors.[9] In keratinocytes and fibroblasts an intracellular receptor for 1,25-DCC seems critical for the metabolic effect of this drug.[10] Alternatively, calcitriol might normalize differentiation of keratinocytes in stable plaques and only indirectly affect recruitment of cells into the cell cycle.

Another explanation might be that the antiproliferative effect of calcitriol, as observed *in vitro* and in animal experiments,[4,5] is not the antipsoriatic mechanism of these drugs. An effect of these drugs on the immune system has been described. For example, interleukin-1 and interleukin-2 induced murine thymocyte proliferation is inhibited substantially by 1,25-DCC.[11] An effect of 1,25-DCC on polymorphonuclear leukocytes might be hypothesized. In the past vitamin D_2 has been reported to be effective in pustular psoriasis.[12]

Another possible explanation for the discrepancy is that calcitriol has only a placebo effect on the psoriatic lesion. Until now only open studies on the effect of these drugs in psoriasis have been carried out; controlled double blind studies are urgently needed.

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Epithelial cutaneous lesions induced in Dunkin-Hartley albino guinea-pigs by means of 7,12-dimethyl-benzanthracene

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SUMMARY

We carried out a clinicopathological study of epithelial cutaneous lesions induced in Dunkin-Hartley albino guinea-pigs by means of the topical application of 7,12-dimethyl-benzanracene. By the end of the study we had observed 4451 lesions. Most frequently observed were epithelial hyperplasia and dysplasia (2544 lesions). Neoplasias consisted of 244 carcinomas *in situ*, and 88 squamous carcinomas. Basal-cell epitheliomas were few. Epidermal cysts were a frequent finding.

Epithelial tumours frequently occur in humans and, therefore, their induction in animals is of considerable interest in order to obtain a greater knowledge of the pathogenesis and biological behaviour of the lesions.

During the last 50 years diverse experimental models have been used employing different animal species and various agents, with very variable results. These studies are justified, however, since any increase in understanding of the mechanism of action of carcinogens, and of the part they play in the initiation of spontaneously arising human neoplasias, may suggest procedures for diminishing or inhibiting their effects.

Guinea-pigs have shown little spontaneous tumoural pathology,[1] although they do show a high degree of sensitivity to polycyclic aromatic hydrocarbons administered subcutaneously, and subsequently develop numerous mesenchymal lesions.[2–5] However, as a species, they apparently are resistant to these agents when applied topically; few cutaneous lesions of an epithelial nature have been successfully induced, lesions of melanocytic origin being a much more frequent result.[6–9]

In the present study we developed a model of epithelial carcinogenesis in Dunkin-Hartley guinea-pigs by means of the cutaneous application of 7,12-dimethyl-benzanthracene (DMBA).

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METHODS

We used 60 2-month-old male Dunkin-Hartley albino guinea-pigs. To each animal 0·3 ml of a 1% solution of 7,12-dimethyl-benzanthracene in acetone (Sigma Chemical Co., St. Louis, Mo, U.S.A.), was applied dropwise from an insulin syringe, to a shaved 5 × 5 cm area of dorsal flank, twice a week for 13 consecutive months.

During the DMBA application phase 20 guinea-pigs died and these were excluded from the study. The development of lesions was followed by clinical observation of the animals and by regular biopsies every 4 weeks. All the guinea-pigs were killed every 2 months in small homogeneous groups during the 17 months after stopping the application of the carcinogen.

Representative samples of the different cutaneous and visceral lesions were fixed in 10% buffered formol and embedded in paraffin by routine methods. Multiple 5 μm sections were cut and stained with haematoxylin and eosin.

RESULTS

By the end of the study 4451 lesions had developed. All the animals were affected to some degree. Lesions are listed here in order of decreasing frequency of presentation.

Hyperplasia

All animals were affected by hyperplasia. This was the first type of change to appear, being observed from the 4th month after the start of the experiment. It was found at 2544 epidermal

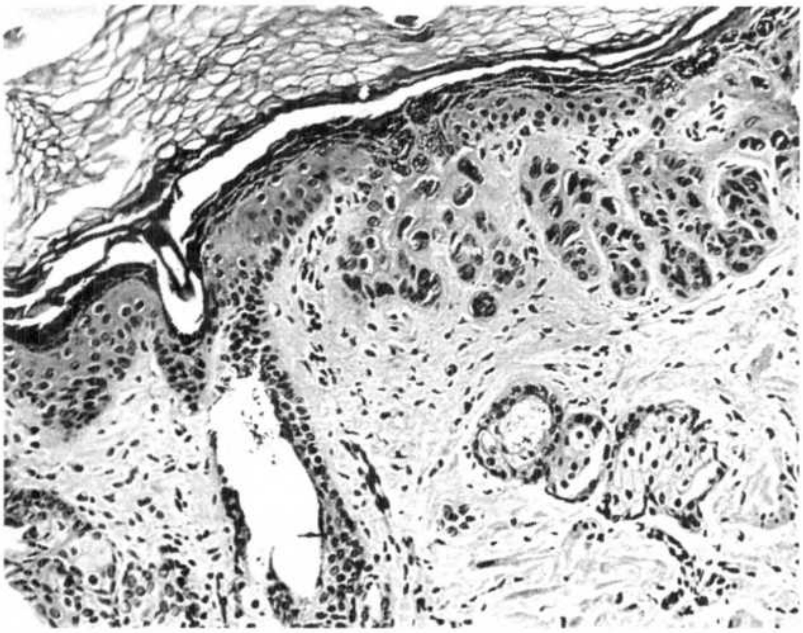

FIGURE 1. Epithelial dysplasia: irregular acanthosis with pleomorphism and hyperchromatic nuclei. Slight dermal lymphocytic infiltrate. (Original × 312·5).

sites. Clinically the appearance was variable, ranging from areas of thickening to areas which were slightly erosive, similar in colour to unaffected adjacent areas. Microscopically there was acanthosis, generally intense, and a variable degree of papillomatosis and hyperkeratosis.

Dysplasia

Dysplasia also affected all animals and was observed from the 4th month onwards. The lesions showed mild to moderate dysplasia. These lesions were observed continuously both in biopsies and in necropsies until the end of the study. Clinically, they were papular lesions of variable size and with irregular edges, sometimes erythematous, sometimes predominantly erosive.

Microscopically the full spectrum of dysplasic change was observed from mild dysplasia, which was observed most frequently, to lesions exhibiting severe dysplasia which were usually accompanied by dermal lymphoplasmacytic infiltrates (Fig. 1). These lesions were found almost always in the epidermis adjacent to squamous cell carcinomas.

Carcinoma in situ

Two hundred and forty-four lesions were found affecting 75% of the animals. They were observed from the 8th month onwards. Clinically they were of two types: squamous papules with irregular edges, and erosive lesions, usually small, with a maximum diameter of between 2 and 4 mm. Microscopically, they showed epithelial thickening which raised the epidermis and also projected towards the dermis. Some lesions showed irregular papillomatosis and also acantholysis with the formation of intraepithelial clefts. Dermal lymphoplasmacytic infiltrates were always present (Fig. 2).

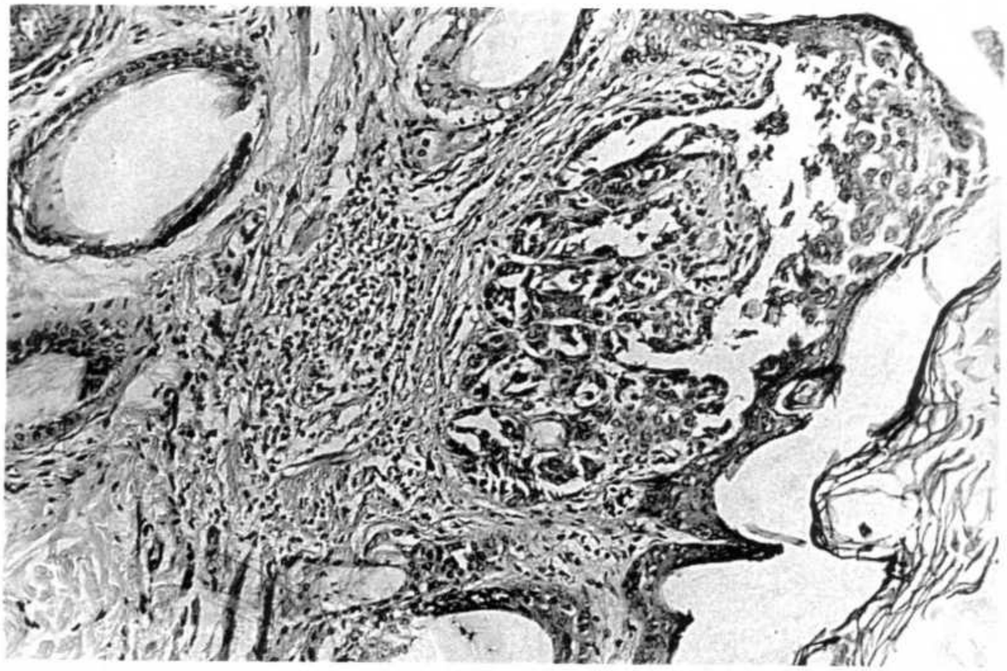

FIGURE 2. Carcinoma *in situ*: marked atypia and a dense dermal lymphocytic infiltrate. (Original × 312·5).

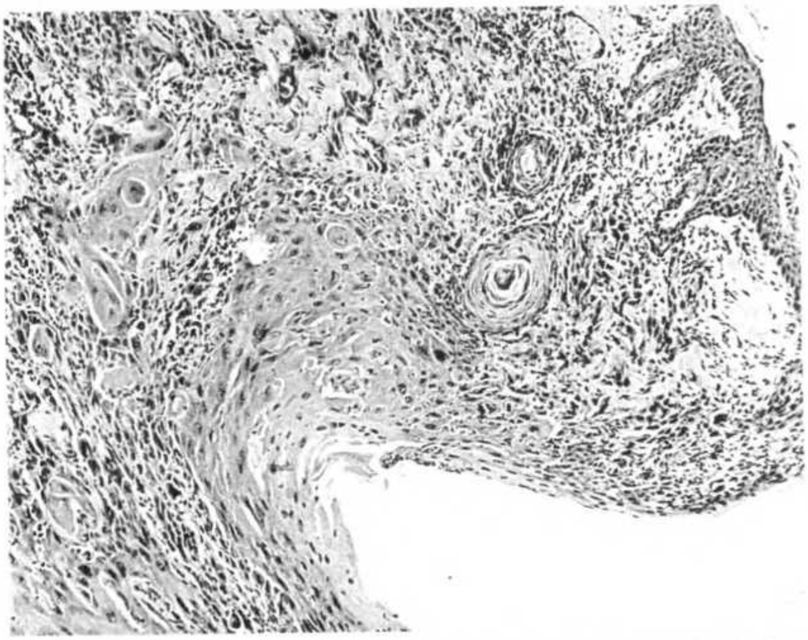

FIGURE 3. Squamous cell carcinoma: neoplastic cellular strands in the dermis, with the formation of frequent horn pearls. Dense lymphoplasmacytic infiltrate. (Original × 312·5).

Squamous cell carcinoma

Eighty-eight lesions were observed. They affected 55% of the animals and were observed from the 12th month onwards, except for two cases which were observed in the 10th month. In some animals multiple lesions of squamous cell carcinoma occurred at a single cutaneous site, separated by narrow bands of unaffected epidermis. Clinically the lesions were of four well-defined types, although sometimes the characteristics of more than one type could be seen in the same lesion. The four types were: vegetants, with a keratotic appearance and of varying size and outline (this was the most numerous type, accounting for almost half of the lesions); ulcerated, the next most numerous and the largest; nodular, with a rough surface and a well-defined outline; papular, whitish in colour with irregular edges. The last two forms were infrequent. Sizes were extremely variable, ranging from 1 to 6·2 cm maximum diameter.

Microscopically the lesions could be divided into three groups according to the degree of differentiation and the state of development of the lesion. About 60% of the lesions were well differentiated. These ranged from vegetant and keratotic lesions, to a few of the ulcerated lesions, and, very exceptionally, to papular or nodular lesions. They frequently showed dyskeratosis and horn pearls (Figs 3 and 4). About 30% of the lesions were moderately differentiated; these included all four clinical types described above although ulcerated lesions predominated. The third group comprised poorly-differentiated lesions, the least frequent type accounting for 10% of the lesions. The majority were ulcerated and the invasion was usually deeper, frequently reaching the subcutaneous tissue and even the underlying muscular tissue. The presence of epithelial strands and nests was rare, as was the occurrence of horn pearls; however, dyskeratosis was observed as in the other types. The predominant cellular pattern was of fusiform cells with elongated and hyperchromatic nuclei with irregular outlines, arranged in

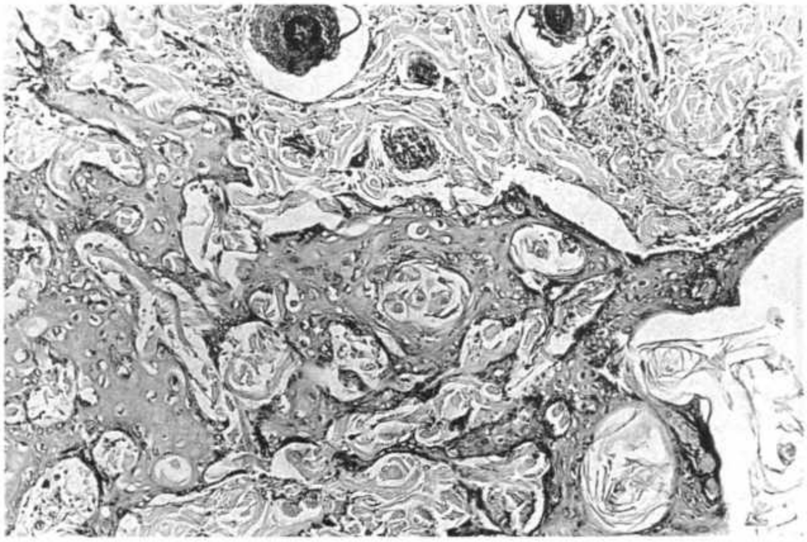

FIGURE 4. Well-differentiated squamous cell carcinoma. Anastomosing epithelial strands with dyskeratosis. (Original × 312·5).

compact bundles in different orientations. In some cases numerous mitoses, signs of vascular permeation, and foci of tumour tissue separated by collagenous or fibromuscular bands were observed. The stroma was of irregular extent, with sparse collagenous fibres and lymphoplasmacytic infiltrates.

Basal-cell epithelioma

Only six basal cell epitheliomas were observed, affecting 10% of the animals. They were observed from the 14th month onwards although most occurred towards the end of the study. Clinically, they were papular pearly lesions, except for one which had a central ulceration and raised irregular edges. All the lesions were small with a maximum diameter of between 2 and 4 mm.

Microscopically, the lesions invaded the papillary dermis, and in one case reached the reticular dermis, but none reached the subcutaneous tissue (Fig. 5). It was frequently necessary to examine a series of sections in order to observe the origin of the neoplasia from the overlying epidermis. Mitoses were infrequent. Occasionally, individual cell keratinization was seen. Between the nests, and almost continuously surrounding all the neoplasia, there was a dense fibrous stroma with irregularly distributed patches of a mild lymphoplas-macytic infiltrate.

Epidermal cysts

Epidermal cysts were observed in 65% of the animals from the 6th month onwards. There were 103 lesions, sometimes several appearing in a single small area. Clinically they were raised nodular lesions varying in size between 0·2 and 1·2 cm. On section the lesions were located in the reticular dermis, exhibiting a cavity filled with whitish chalky-looking material. Histologically, the wall was made up of well-differentiated, stratified, flat epithelium (Fig. 5). Horny material, arranged in concentric lamellae, filled the lumen.

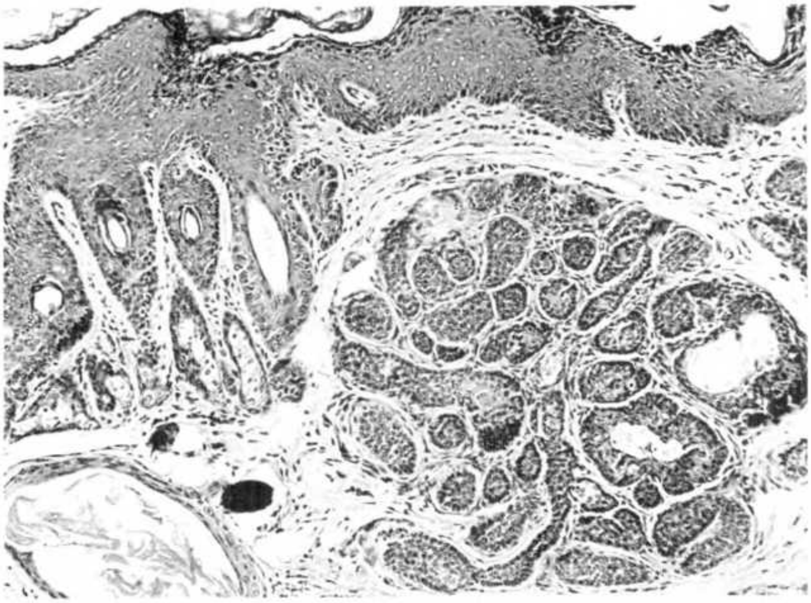

FIGURE 5. Basal cell epithelioma: Nodular lesion and epidermal cyst on the lower left. (Original × 312.5).

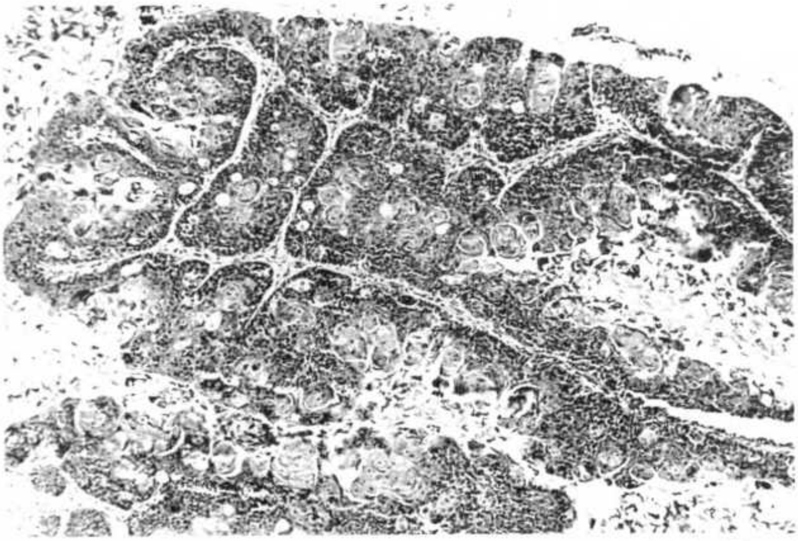

FIGURE 6. Proliferant trichilemmal cyst: Epithelial strands with frequent individual cell keratinization. (Original × 312·5).

Proliferant trichilemmal cyst

One lesion of this type was found near the end of the study. Clinically, it was nodular, elevated and 0·6 cm maximum diameter. On section, the lesion exhibited a cavity with small irregular papillary formations towards the lumen, and appeared to be filled by keratotic greasy-looking material. Histologically, the wall was made up of stratified flat epithelium with cellular vacuolization. In the papillae, cellular atypia, dyskeratosis and horn pearls were frequent (Fig. 6). The lumen was filled with compact homogeneous, eosinophilic material with occasional foci of calcification.

Cutaneous atrophy

This lesion was rare, being found in only six treated areas and in the last months of the study. Clinically, the affected areas were smooth and hairless. Histologically, the epidermis was made up of only two or three layers of keratinocytes with flattening of the rete-ridges, hyperkeratosis and absence of hair follicles and sebaceous glands. In the dermis there was slight fibrosis in the papillary layer with small perivascular lympohistiocytic infiltrates.

DISCUSSION

Guinea-pigs are an ideal animal model for induced cutaneous carginogenesis since, as Rogers *et al.*[1] point out in their extensive bibliographical survey, only 0·4% of the 4000 animals studied showed spontaneous neoplasias; almost all of these lesions were mesenchymal in origin and only one was epithelial.

In addition, guinea-pigs have a high sensitivity to the action of diverse chemical agents.[5] They are extremely sensitive to alkylants and nitrosamines; to aflatoxins, which produce hepatic carcinogenesis, and to aromatic amines which induce urinary tumoural pathology and mammary and ovarian tumours. Nevertheless, these facts conflict with the opinion widely held until the 1960s that guinea-pigs showed an inherent resistance as a species to chemical carcinogenesis. This was because the results obtained until then had been inferior to those obtained with other rodents. Those results were probably due to the mode of application of the carcinogen since the polycyclic hydrocarbons most utilized in experimental carcinogenesis were tried, including benzopyrene, which was applied subcutaneously by Berenblum,[10] Haranghy *et al.*,[11] and Maltoni, Laschi and Carolo;[12] the latter authors only managed to produce mesenchymal lesions and a total of three squamous carcinomas.

In previous models[7,8,13,14] epithelial lesions tended to receive little attention since the results obtained were variable and few neoplastic lesions appeared. This also seems to have been the case in earlier studies.[15,16]

We consider the experimental model of carcinogenesis developed in the present study to be of interest since, as well as the epithelial lesions which developed in all animals, melanocytic lesions and occasional mesenchymal lesions were also produced, although the latter two lesion types were not considered relevant to the present study and are not presented in this report.

The total number of lesions, with 2544 areas of epithelial hyperplasia and 1433 of epithelial dysplasia which affected 100% of the animals and were observed from the 4th month after the starting of the experiment, is in sharp contrast to the numbers of these lesions found in other experimental guinea-pig models; Berenblum[13] only mentions one papilloma and one squamous carcinoma; Edgcomb and Mitchelich[14] found papillomas in 50% of their animals, but no cases of malignant neoplasia; Pawlowski, Haberman and Menon[8] indicated the frequent appearance of papillomas, but only reported four cases of squamous carcinoma.

In contrast, we found in 75% of the animals from the 8th month onwards 234 areas with carcinoma *in situ*, and from the 10th month onwards we observed 88 squamous carcinomas, mainly well differentiated and affecting 55% of the animals.

Whilst none of the above authors described the presence of basal cell epitheliomas we observed six lesions of this type in four guinea-pigs, from the 14th month. If these lesions originate in the cells of the external layer of the hair follicle, their infrequent occurence might be explained by the fact that these cells are protected from the action of the carcinogen. We believe, however, that the most likely explanation for the paucity of lesions of this type (which in human pathology are the most frequently occurring cutaneous neoplasias) could be the length of time needed for their development. The long development period of multicentric basal cell epithelioma in human pathology is well-known, and all models previously used were observed for periods less than 2 years.

Very frequently lesions, occurring in 65% of our animals in 103 different areas, were epidermal cysts. Edgcomb and Mitchelich[14] found these in 50% of their animals and Pawlowski, Haberman and Menon[8] also described them as frequent lesions. Their pathogenesis is unclear. It could represent an intermediate change between dysplasia and true neoplasia. As a curiosity, we have presented one case of a proliferant trichilemmal cyst of which we have found no mention in any previous experimental model described. Its pathogenesis could be similar to that of epidermal cysts.

One of our findings which is in stark contrast to the results of Edgcomb and Mitchelich[14] and Pawlowski *et al.*[8] concerns the presence of cutaneous atrophy; these authors describe it as a very frequent finding, indeed as almost constant, whereas we found it in only 10% of our animals. We believe our result is closer to what would be expected in a model of cutaneous carcinogenesis.

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Ketanserin in the treatment of Raynaud's phenomenon associated with generalized scleroderma

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SUMMARY

The efficacy of the 5-HT-2-receptor antagonist, ketanserin, in the treatment of Raynaud's phenomenon was assessed in a double-blind, placebo-controlled, crossover trial in nine patients with generalized scleroderma (GS). Each patient received ketanserin 20 mg or placebo three times a day in the 1st week and 40 mg ketanserin or placebo three times a day for the remaining 4 weeks, and was then crossed over for 5 weeks. Measurements were made of finger blood pressure and flow during a cold challenge test and patients recorded numbers of Raynaud's attacks.

No significant improvement was found in reactions to cold provocation or in numbers of Raynaud's attacks during ketanserin treatment, but intolerable side-effects were common. Interactions with concomitant medication might be a possible explanation for this. We conclude that ketanserin in the dose given, is not effective in the treatment of Raynaud's phenomenon in GS.

Generalized scleroderma (GS) is accompanied by Raynaud's phenomenon in over 95% of cases.[1] The precise mechanism of intermittent finger ischaemia in GS is unknown, and no drug therapy is universally effective or tolerated.[1] However, serotonin (5-HT) is suggested to promote the phenomenon in GS by causing smooth muscle contraction and potentiating platelet aggregation.[1,2]

Stranden *et al.*[3] showed that injections of the 5-HT-2-receptor antagonist, ketanserin, into the brachial artery transiently improved digital blood flow in patients with Raynaud's phenomenon, and Seibold *et al.*[1] and Roald and Seem[4] reported the value of this drug when given orally, the best results being obtained in GS.

In the present study we have evaluated the effects of ketanserin on the symptoms of Raynaud's phenomenon in patients with GS in a double-blind, placebo-controlled, crossover trial.

METHODS

Nine patients (six women and three men, median age 45 years; range 30–70) with GS and

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Raynaud's phenomenon entered the study. Following 2 weeks of placebo treatment (run-in period), the patients were assigned randomly to receive either placebo or ketanserin (Jansen) 20 mg three times per day in the 1st week and 40 mg three times per day for the remaining 4 weeks. A second 5-week treatment period with the alternative medication followed without an intervening washout period. Each day the patients filled in diary sheets with the number of Raynaud's attacks and their subjective feelings.

On the 1st day of the trial, after the run-in period and again after each of the two treatment periods, the patients underwent a cold challenge test and a physical examination.

Cold challenge test

Patients were tested in the supine position, lightly dressed and lying on a water-perfused blanket. For measurement of finger systolic pressure (FBP) a water perfused finger cuff was used ('digit cooling' system, Medimatic, Birkerod, Denmark). A laser Doppler flowmeter (Perimed, Stockholm, Sweden) was used to measure skin blood flow in the fingertip, including flow during FBP measurements.[4] FBP was measured after 15 min warming of the patients on a 30°C blanket and the finger "cooled" to 30°C, and repeated after 15 min cooling on a 15°C blanket and the finger cooled to 15°C. Results were expressed as percentage laser Doppler blood flow and FBP at 15°C relative to readings at 30°C. The duration of the induced vasospasm and decreased blood flow were also recorded.

As an inclusion criterion for the study, the % decrease in FBP during coding challenge was set at >60% and all patients had normal kidney and liver function tests. Blood samples for measurement of plasma ketanserin levels were taken after each of the two treatment periods, approximately 2 h after the last dose of the drug.

Statistics

A Wilcoxon matched pairs signed ranks test was used to compare results for ketanserin and placebo. Regression analysis was used to estimate the correlation between plasma ketanserin levels and objective measurements; 0·05 was chosen as a limit of significance in both tests.

RESULTS

The results are summarized in Table 1. No significant increase in finger blood flow or FBP during cold challenge was observed during treatment with ketanserin. However, a reduction of the duration of induced vasospasm was observed, but this improvement was not statistically significant. There was no significant difference in the numbers of attacks recorded in the patients' diaries (Table 1) and no effect on healing of finger ulcers was observed (Table 1). Intolerable side-effects were common.

The correlation coefficients between plasma ketanserin levels and each of the three objective parameters were as follows: laser Doppler flow $r = -0{\cdot}57$; FBP $r = 0{\cdot}54$; and duration of vasospasm, $r = 0{\cdot}49$. None of these correlations was statistically significant.

DISCUSSION

The same dose of ketanserin as we used in the present study has been well tolerated in earlier trials.[1,4] Interaction with concomitant drug therapy might be a possible explanation for the severe adverse effects observed in the present study.

All patients received penicillamine. This might have interfered with plasma levels of

TABLE 1. Results of cold provocation testing, numbers of Raynaud's attacks and side effects in nine patients with generalized scleroderma while receiving ketanserin or placebo

Patient No.	Concomitant medication	Cold provocation testing*			No. of Raynaud's attacks as % of times outside	Side effects		Plasma ketanserin level ng/ml	Effect on finger ulcers
		Laser doppler flow (% change)	FBP (% change)	Duration of attack (min)		Ketanserin	Placebo		
1	Penicillamine	R 23	0	0·3	81	Weight gain, tired, dizzy,	Felt generally		
	Glutamine	P 27	42	0	29	vomiting, diarrhoea,	better, warmer	85	
	Mianserin	K 14	0	0·5	0	colder hands	hands		
2	Penicillamine	R 7	0	3·5	91	Weight gain			
	Glutamine	P 15	0	3·5	82			65	
	Cimetidine	K 9	0	3·5	82				
3	Penicillamine	R 7	0	4·0	94	Tired, burning skin.			
	Glutamine	P 16	0	4·0	83	Stopped after week 10		170	
	Ranitidine	K 27	0	2·5	85				
	Furosemide								
4	Penicillamine	R 0	0	4·0		Dizzy	Dizzy, bleeding		
	Glutamine	K 0	0	4·0	Too low to		oesophagitis,	80	
							Decrease in		
							Arm blood pressure		
		P 0	0	Constant 'attack'	analyse		BP		
5	Penicillamine	R 0	0	4	82	Weight gain, leg oedema,			Healed on
	Glutamine	P 100	0	4	63	dry skin with fissures		200	placebo
	Cimetidine	K 6	47	0	51				
6	Penicillamine	R 16	0	1	100	Oedema of fingers and legs,			
	Glutamine	K 20	40	0	100	dizzy, diarrhoea		140	
	Amoxacillin (week 6)	P 47	67	0	not recorded				
7	Penicillamine	R 58	44	0	45	Weight loss, oedema of			Unchanged
	Glutamine	K 100	25	0	24	fingers and legs,		40	
		P 112	47	0	23	dry mouth			
8	Penicillamine	R 49	55	0					Unchanged
	Glutamine	K 70	100	0	not recorded			<4	
	Penicillin (week 3)	P —	—	—					
9	Penicillamine	R 8	0	3·0	60	Dry mouth,	Painful, tight		
	Glutamine	K 60	0	1·3	50	flaccid leg muscles	leg muscles.	75	
	Oestrogen/	P 0	0	2·0	30		Stopped after		
	Progesterone						week 10		
	Diazepam								
	Chlorzoxazon (week 6)								
	(muscle relaxing)								
Median		R 8(0–58)	0(0–55)	3(0–4)					
(Range)		P 27(0–112)	0(0–67)	2(0–4)					
		K 20(0–100)	0(0–100)	0·5(0–4)					

*R = end of run in; P = placebo; K = ketanserin; results underlined.

ketanserin, as both drugs are highly bound to plasma proteins or interfered with the clearance, as both drugs are excreted by the kidneys. Although no clear relation could be observed between adverse reactions and plasma ketanserin levels, this would appear to be the most probable explanation. Ketanserin has a high affinity for S-2-receptors in blood vessels and platelets, antagonizing the vasoconstricting and platelet aggregating effect of serotonin. Although all the effects of penicillamine are not known, it appears unlikely that it should interact with ketanserin at its site of action.

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Acitretin and etretinate in the treatment of palmoplantar pustulosis: a double-blind comparative trial

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SUMMARY

Sixty patients with palmoplantar pustulosis were treated in a double-blind trial with either acitretin (etretin, Ro 10-1670) or with etretinate. The study consisted of 4 weeks of therapy with three 10 mg capsules/day followed by 8 weeks of therapy with a varying number of capsules given daily according to therapeutic response. At the end of the 12-week treatment period, the mean number of pustules (± SEM) had decreased from 57·8 (± 8·6) to 3·9 (± 1·6) in the acitretin group and from 57·1 (± 14·1) to 5·7 (± 2·7) in the etretinate group. With regard to influence on erythema, infiltration, scaling, and area involved, similar improvements were obtained in both treatment groups. Adverse reactions of the hypervitaminosis A type were observed with almost the same frequency and severity in both treatment groups. The mean number of 10 mg capsules used daily was comparable in the two groups: 2·82 (range 1·23–4·67) for acitretin and 2·77 (range 1·60–4·82) for etretinate. It can be concluded that acitretin and etretinate do not significantly differ with regard to efficacy and overall safety in the treatment of patients with palmoplantar pustulosis.

Acitretin (Ro 10–1670, etretin) is the acid form of the ethyl ester etretinate. During treatment with etretinate, Ro 10-1670 is formed by hydrolysis in the body and is considered to be the main active principle of the drug. It was recently shown that acitretin is effective in the treatment of severe psoriasis[1,2] and various disorders of keratinization.[3,4] An important advantage of acitretin over etretinate is its much faster elimination from the body.[5]

Palmoplantar pustulosis (PPP) is a chronic disease characterized by pustular eruptions and erythema with scaling which can be cleared or markedly improved by etretinate treatment.[6–15] The aim of the present study was to compare the therapeutic effects of acitretin and etretinate in PPP, using a randomized, double-blind trial design.

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METHODS

Sixty patients with PPP were recruited to participate in the study. Patients with severely impaired renal or hepatic function or with severe cardiological or neurological disease were excluded. Women of childbearing age were only included if they were using reliable contraception. Patients who had received etretinate within the 6 months prior to the trial and patients recalcitrant to previous treatment with etretinate were excluded. Patients who had received PUVA, topical corticosteroids, or other active treatment of PPP within the 4 weeks prior to the trial were also excluded. Patients were fully informed about the risks of the treatment, and all gave verbal consent to participation in the study.

Eighteen men and 42 women, age range 22–76 years, were randomly allocated to one of the two retinoid treatment groups (Table 1).

Capsules containing 10 mg acitretin or 10 mg etretinate, identical in size and colour, were used in the study. During the first 4 weeks, the patients received three capsules per day. This dose was chosen on the basis of previous studies in psoriasis.[2,3] During the following 8 weeks, the number of capsules was individually adjusted according to clinical improvement and tolerance. The capsules were taken once daily, during the main meal.

Patients were examined before treatment and at weeks 2, 4, 8 and 12. At each examination the number of fresh pustules was counted on the right and left palms and soles. Intensity of erythema, infiltration and scaling were scored on a four point scale (severity score) where 3 = severe, 2 = moderate, 1 = mild and 0 = none. The area of involvement on each palm or sole was estimated visually to the nearest 10% and the mean of the two sides was calculated.

At each follow-up visit, all patients were examined and questioned about adverse effects. Blood samples were taken before treatment and at 4-week intervals for measurement of gamma-GT, SGOT, SGPT, cholesterol and triglycerides.

Statistical analysis of the numbers of pustules and the involved area on the palms and soles was performed using the Mann-Whitney U-test. For intensity of erythema, infiltration and scaling, the treatment groups were compared using the two sided Krauth-test (exact linear rank-test, Stucky-Vollmar procedure).

RESULTS

Two patients left the study before 12 weeks of treatment; one from the etretinate group failed to return for follow-up visits after the week 2 check while the other patient, who belonged to the acitretin group, discontinued treatment because of complete remission after 4 weeks.

The mean number of 10 mg capsules taken daily by the patients over the 12 week period was effectively identical in the acitretin group (mean: 2·82; range: 1·23–4·67) and the etretinate group (mean: 2·77; range: 1·60–4·82). Topical corticosteroids, local antiseptics or oral antibiotics were temporarily used as additional treatment for inflammation or superinfection in six patients in the acitretin group and four patients in the etretinate group.

A similar reduction in the mean number of fresh pustules per patient (± SEM) was obtained in both treatment groups; from 57·8 (± 8·6) to 3·9 (± 1·6) in the acitretin group and from 57·1 (± 14·1) to 5·7 (± 2·7) in the etretinate group (Table 2). The numbers of pustules in the two groups were not significantly different before treatment ($P > 0{\cdot}5$) or at the end of treatment ($P > 0{\cdot}5$). With regard to changes in the scores for erythema, infiltration and scaling, no significant differences between the two treatment groups was found. There was no statistically significant difference between treatment groups in the number of patients with different degrees

TABLE 1. Clinical details of 60 patients who took part in the study

	Acitretin	Etretinate
No. of patients	30	30
Males	11	7
Females	19	23
Age (years); mean (range)	49·9 (22–70)	52·9 (27–76)
Weight (kg); mean (range)	69·9 (51–85)	70·1 (46–100)
Height (cm); mean (range)	167·9 (155–186)	166·3 (153–190)
Number of patients with		
lesion on palms	24	22
on soles	20	23

TABLE 2. Numbers of pustules, scores for erythema, infiltration and scaling and area of palms and soles involved in the two groups of patients Values are means ± SEM

					Area involved (%)	
	Pustules	Erythema	Infiltration	Scaling	Palms	Soles
Acitretin		*n* = 29 or *28			*n* = 24 or *23	*n* = 19
Baseline	57·8 ± 8·6	2·76 ± 0·09	1·86 ± 0·08	1·66 ± 0·11	32 ± 5	32 ± 4
Week 2	16·5 ± 11·1	2·00 ± 0·14	1·03 ± 0·09	1·00 ± 0·15	28 ± 6	31 ± 5
Week 4	16·4 ± 7·7	1·62 ± 0·16	1·00 ± 0·12	1·21 ± 0·14	25 ± 6	28 ± 5
Week 8	*9·2 ± 2·8	*1·07 ± 0·17	*0·75 ± 0·13	*0·86 ± 0·13	*11 ± 4	27 ± 4
Week 12	*3·9 ± 8·5	*0·93 ± 0·17	*0·55 ± 0·11	*0·69 ± 0·14	*4 ± 2	19 ± 4
19+4						
Etretinate		*n* = 29 or *28			*n* = 21 or *20	*n* = 22 or *21
Baseline	57·1 ± 14·1	2·55 ± 0·14	1·86 ± 0·08	1·59 ± 0·11	30 ± 4	39 ± 5
Week 2	*17·2 ± 8·3	*1·79 ± 0·15	*1·39 ± 0·12	*1·21 ± 0·16	*26 ± 4	*34 ± 5
Week 4	11·5 ± 5·1	1·52 ± 0·14	1·03 ± 0·13	1·07 ± 0·17	20 ± 5	29 ± 6
Week 8	7·2 ± 3·1	1·03 ± 0·18	0·90 ± 0·14	0·72 ± 0·15	10 ± 3	17 ± 4
Week 12	5·7 ± 2·7	1·07 ± 0·22	0·72 ± 0·13	0·62 ± 0·04	9 ± 4	17 ± 3

of severity of erythema, infiltration and scaling (before or after treatment ($P > 0·3$). All these three clinical features showed a similar marked improvement with both acitretin and etretinate.

Before treatment, the areas of palms and soles affected were comparable in the two groups ($P > 0·5$). At the end of therapy, there was no statistically significant difference between the two groups ($P > 0·1$). However, palmar lesions responded better to retinoid treatment than did plantar lesions.

In both treatment groups, all the patients experienced adverse reactions of the hypervitaminosis A type, as listed in Table 3. The frequency of adverse reactions observed in the acitretin group tended to be higher than that in the etretinate group.

Overall, the same number of adverse reaction (9) were considered by the patients to be troublesome in both groups during the whole treatment period.

TABLE 3. Clinical adverse events observed during the whole treatment period in the two groups

	Acitretin ($n = 30$)		Etretinate ($n = 29$)	
	No. of patients	%	No. of patients	%
Dry lips/cheilitis	30	100%	29	100%
Dry mouth	22	73%	18	62%
Dry skin	21	70%	12	41%
Dry nose	18	60%	10	34%
Scaling (palms/soles)	18	60%	14	48%
Scaling (elsewhere)	14	47%	7	24%
Dry eyes/conjunctivitis	6	20%	7	24%
Hair loss	6	20%	4	14%
Pruritus	3	10%	2	7%
Nail fragility	2	7%	2	7%
Other	7**	23%	1***	3%

** Localized infection 2, erythema 1, retinoid dermatitis 1, purpura 1, dysuria 1, nausea 1.

*** Sticky skin 1.

No laboratory abnormalities were observed during the study. In particular, serum transaminases and lipids showed no marked elevations in any of the patients.

DISCUSSION

In previous clinical studies, etretinate proved to be significantly more effective than placebo[7,11,12,14] or PUVA[13] in the treatment of PPP. Recently, it was reported that the combination of etretinate and PUVA should be the treatment of choice for PPP.[15] In these studies, intolerable adverse reactions were reported in many patients when etretinate was administered at high doses of about 1 mg/kg/day.

A great disadvantage of etretinate is its very slow elimination from the body and, owing to its teratogenic potential, pregnancy must be avoided until at least 2 years after stopping the drug. Acitretin has a much shorter elimination half-life and women may be permitted to become pregnant far sooner following treatment than is now the case for etretinate.

In the present double-blind study in patients with PPP, we compared the new drug acitretin with its parent drug etretinate. The results indicate that both retinoids were equally effective. Similar reductions in pustule counts and area affected were found, and intensity of erythema, infiltration and scaling decreased in the same manner in both treatment groups.

The adverse reactions in both treatment groups involved almost exclusively the mucous membranes and the skin. They were easily controllable by decreasing the dosage. This good tolerance, compared with that in previous studies with etretinate,[6–15] can be explained by the lower doses of retinoids administered to our patients. Taking into account both efficacy and tolerance, the optimal dose for both retinoids was approximately 30 mg/day, which is less than half the dose of 1 mg/kg/day used previously.[15]

It can be concluded from this study that acitretin is effective in the treatment of PPP, and that efficacy and tolerance of acitretin and etretinate are not significantly different.

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The use of antipyrine clearance to measure liver damage in psoriatic patients receiving methotrexate

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SUMMARY

Salivary antipyrine clearance was measured in 15 patients with psoriasis receiving methotrexate and related to liver biopsy changes and routine liver function tests, to determine whether antipyrine clearance could be used as a non-invasive method for monitoring liver function. Comparison of the salivary antipyrine clearance in the methotrexate group (mean 0·51 ml/min/kg, range 0·26–0·81) with 15 matched psoriatic controls (mean 0·54 ml/min/kg, range 0·34–0·99) showed no significant difference. Liver biopsy changes were scored for fatty change, fibrosis, liver cell necrosis and portal tract infiltrate. Total liver biopsy scores correlated significantly with antipyrine clearance/kg body weight ($r = -0{\cdot}72$, $P < 0{\cdot}05$). There was no significant correlation of total liver biopsy scores with the other liver function tests. Correlation of the individual liver biopsy changes and antipyrine clearance showed a significant correlation with fatty change ($r = -0{\cdot}75$, $P < 0{\cdot}01$), but not with fibrosis ($r = -0{\cdot}53$), liver cell necrosis ($r = -0{\cdot}54$) or infiltrate ($r = -0{\cdot}41$).

Liver biopsy is the only reliable method available at present for monitoring liver damage in patients taking methotrexate for treatment of psoriasis. This invasive procedure requires hospital admission and may not always provide adequate information.[1] Non-invasive methods of assessing liver damage including conventional liver function tests, bile salt estimations,[2] ultrasound,[3] technetium-99 sulphur colloid isotope scans[4] and magnetic resonance imaging[5] have all been tried and proved to be indequate in assessing hepatic damage.

Antipyrine clearance has been widely used to study the pharmacokinetics of drug metabolism and has also been suggested as a method of monitoring liver functions.[6] In the context of assessing patients with psoriasis, antipyrine clearance measurements may be useful since clearance is significantly reduced in both patients with psoriasis[7,8] and rats[9] after methotrexate administration. We have studied the relationship between antipyrine clearance and liver

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damage in psoriatic patients receiving methotrexate, to determine whether this test could be used as a non-invasive method of monitoring liver damage.

METHODS

Patients

Thirty patients with psoriasis were studied. These included 15 patients (six females and nine males, mean age 48·3 years, range 31–64 years) receiving methotrexate (mean cumulative dose 2194 mg, range 992–4450 mg) and 15 control patients matched for age (to within 5 years), sex and smoking habits who were not receiving methotrexate. Liver biopsies were performed only on the 15 patients receiving methotrexate. Alcohol consumption in the methotrexate treated group ranged from 0–16 units/week, mean 3·5, and from 0–29 units/week, mean 10·0, in the control patients. Eight of the methotrexate treated patients and nine controls were on other medication. In two patients this medication is known to increase antipyrine clearance[10]: patient 5 was also taking primidone and phenytoin and one control patient was taking spironolactone.

Liver function tests

The plasma concentration of albumin and bilirubin and the activities of alkaline phosphatase (AP), aspartate aminotransferase (AST) and glutamyl transferase (GGT) were measured with a Sequential Multiple Analyser with Computer System (Technicon Instruments Co, Basingstoke, Hants U.K.).

Liver biopsy

Liver biopsy specimens were examined and scored 'blind' by a pathologist. Each biopsy was examined for the degree of fatty change, the number of inflammatory cells per portal tract, the extent of any fibrosis and the extent of liver cell necrosis.[2] Each of these parameters was scored from 0–3 depending on severity and the score for all four features were added to give a total score.

Salivary antipyrine clearance

Antipyrine clearance was measured using serial saliva samples rather than blood because of the ease of collection by the patient at home and the proven accuracy of this method.[11] Saliva flow was stimulated, after an overnight fast, by chewing a piece of Parafilm and a 5 ml sample of saliva was collected. Antipyrine 600 mg (Phenazone) was then given with water and further saliva samples collected 3, 5, 8, 12, 16, 24 and 48 h later. Samples were stored at −20°C. Antipyrine concentration was determined using a colorimetric method.[12]

The concentration of antipyrine at zero time was calculated by extrapolating to the vertical axis a straight line plot of the $\log_{10}$ plasma antipyrine concentration against time. Half-life was the time at which antipyrine concentration was half its theoretical maximum value. The antipyrine clearance was calculated from the following formula.

$$\text{Antipyrine clearance (ml/min/kg)} = \frac{\text{Elimination rate constant (ERC)} \times \text{volume of distribution}}{\text{weight (kg)}}$$

where $\text{ERC} = 0{\cdot}693/\text{half-life}$.

$$\text{Volume of distribution} = \frac{\text{dose given}}{\text{concentration at zero time}}$$

Statistical methods

Antipyrine clearance results in the two groups were compared using an independent t-test (2-tailed). Correlations were measured using Spearman's rank correlation coefficient (2-tailed).

RESULTS

Liver biopsy and liver function tests

The results are shown in Table 1. None of the 15 biopsies examined showed cirrhosis. Twelve biopsies showed fatty change, six had a cellular infiltrate around the portal tract, seven showed fibrotic changes and nine biopsies showed areas of necrosis. Abnormal liver function test results included low albumin concentrations in two patients, raised AP activities in five patients, raised AST activities in two patients and raised GGT activities in five patients; total bilirubin concentration was raised in one patient.

Antipyrine clearance

Antipyrine clearance values were normally distributed in the methotrexate and control groups. The mean antipyrine clearance values in the 15 patients receiving methotrexate and the matched controls were 0·51 ml/min/kg (range 0·26–0·87) and 0·54 ml/min/kg (range 0·34–0·99), respectively. There was no significant difference in antipyrine half-life, concentration at zero time or clearance/kg body weight, between the patients receiving methotrexate and controls.

Comparison of total liver biopsy score with liver function tests and antipyrine clearance

The correlation between the total liver biopsy score and the various liver function tests was

TABLE 1. Liver biopsy scores, liver function tests and antipyrine clearance in patients receiving methotrexate for psoriasis

	Liver biopsy score					Liver function tests*					
Patient no.	Fatty change	Infil-trate	Fibrosis	Necrosis	Total	ALb g/l (39–150)	AP u/l (45–120)	AST u/l (0–40)	GGT u/l (0–35)	Bili µmol/l (0–17)	Antipyrine clearance ml/min/kg
1	2	0·5	1	1	4·5	45	113	44**	—	9	0·30
2	2	1	3	2	8	36**	186**	32	38**	6	0·35
3	3	0	1	2	6	42	95	20	10	5	0·37
4	2	0·5	1	2	5·5	51	59	57**	21	15	0·35
5	0·5	0	0	0·5	0·5	46	151**	26	142**	7	0·72
6	0·5	0	0	0·5	0·5	47	66	18	15	8	0·59
7	1	0	0	0	1	47	68	26	28	5	0·35
8	2	1	0	2	5	42	208**	28	162**	6	0·46
9	2	0	0	2	4	47	87	26	27	19**	0·49
10	2	0	0·5	2	4·5	44	168**	24	23	12	0·52
11	0	1	2	2	5	43	78	23	33	8	0·67
12	2	0·5	2	3	7·5	30**	225**	25	85**	4	0·26
13	0	0	0	0	0	44	54	29	77**	10	0·67
14	0·5	0	0	0	0·5	45	78	19	23	3	0·65
15	0	0	0	0	0	45	87	25	29	7	0·87

* Values in parentheses are normal ranges.

** Levels outside the normal range.

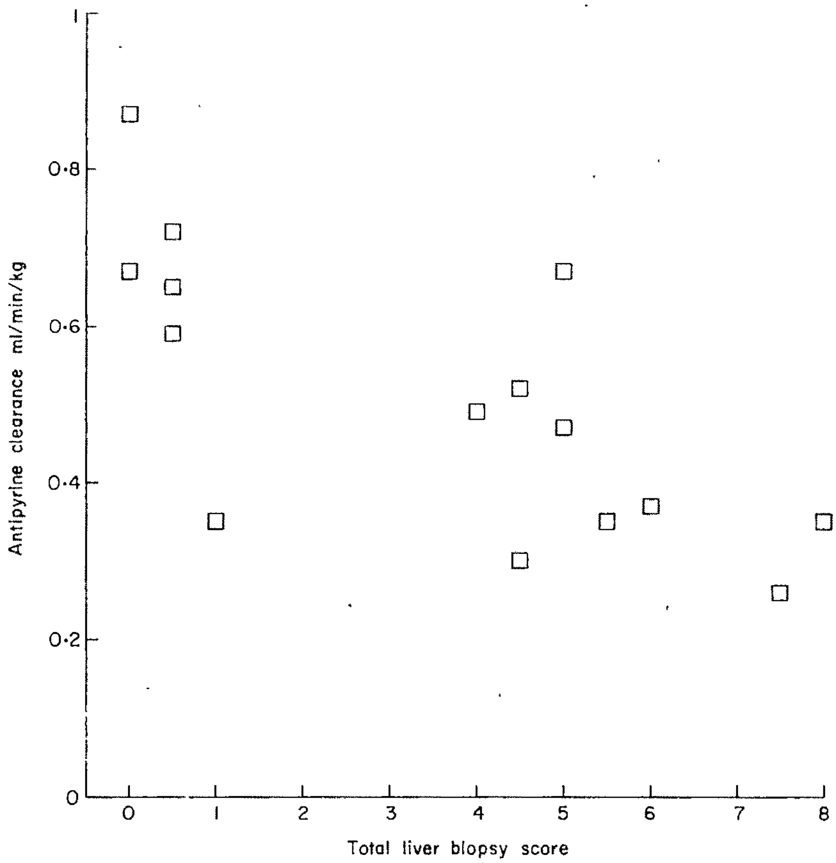

FIGURE 1. Relationship between antipyrine clearance and total liver biopsy score in 15 patients receiving methotrexate for psoriasis.

analysed using the Spearman rank correlation coefficient. Antipyrine clearance/kg body weight showed a significant correlation with the total liver biopsy score ($r = -0{\cdot}72$; $P < 0{\cdot}05$) (Fig. 1). Antipyrine concentration at zero time and antipyrine clearance (uncorrected for body weight), also showed smaller although significant correlations with total liver biopsy score ($r = 0{\cdot}57$ and $r = -0{\cdot}69$, respectively; $P < 0{\cdot}05$). The other liver function tests did not show a significant correlation with total liver biopsy score. Comparison of the individual liver biopsy changes with the antipyrine clearance/kg body weight showed that the highest correlation was with fatty change ($r = -0{\cdot}75$, $P < 0{\cdot}01$). The correlations with infiltrate ($r = -0{\cdot}43$), fibrosis ($r = -0{\cdot}53$), and cell necrosis ($r = 0{\cdot}54$), were not significant.

DISCUSSION

This study shows that antipyrine clearance was the only test of liver function studied that was significantly correlated with liver biopsy scores. Methotrexate therapy did not affect the antipyrine clearance, and values were similar to those obtained in psoriatics by other workers.[7] Long-term methotrexate therapy may cause hepatic fibrosis, cirrhosis or hepatitis.[13] Antipyrine clearance just failed to achieve a significant correlation with the degree of fibrosis or necrosis, but was significantly correlated with the degree of fatty change. Since the most important aspect of the liver biopsy histological appearance is fibrosis and liver cell necrosis, it appears that antipyrine clearance is not an ideal test of hepatic damage in patients receiving long term methotrexate. However, clearance was considerably more useful that the routine liver function tests used and sequential measurements in individuals may provide a simple, non-invasive method of monitoring liver function, provided the effects of cigarette smoking or drug therapy are taken into account.

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The effect of topical cyclosporin on the immediate shedding of human scalp hair grafted onto nude mice

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SUMMARY

Considerable evidence now exists that cyclosporin (CyA) has a stimulatory effect on hair growth. Previously, we demonstrated the systemic effect of CyA on hair growth using an experimental model of human scalp skin graft transplanted onto nude mice and rats. In the present study we used this model to investigate the effect of topical CyA on human hair growth. One group of 15 mice was treated with topical CyA in olive oil, and a second group of 10 mice with olive oil only. The numbers of grafts with hair and the number of hairs per graft in the different groups were compared. A statistically significant delay in hair shedding appeared from day 24 onwards in the CyA treated group. This pilot study supports the possibility that CyA may be effective in the treatment of alopecia.

Cyclosporin A (CyA) has been used primarily in organ transplantation, but it seems that the drug may also have some potential use in disorders of hair growth. Indeed, hypertrichosis is known to be a side-effect of CyA therapy in 30–42% of patients.[1,2] Recently, we demonstrated the systemic effect of CyA on hair growth using a model in which scalp skin grafts obtained from patients with alopecia areata and alopecia universalis were transplanted onto nude mice.[3] We suggested that this murine transplantation model may be useful for the study of drug-induced human hair growth. We have previously shown that topical application of CyA had no side-effects and that blood levels were very low.[4] The aim of the present investigation was to study the effect of topical CyA on the prevention of shedding and on the growth of human hair in the murine transplantation model using scalp skin obtained during elective face lift procedures.

METHODS

Experiments were performed on human scalp grafts transplanted onto nude mice as previously described.[3]

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Animals

Outbred Balb-C nude mice, 2–3 months of age, were used in the study. The animals were obtained from the pathogen free animal breeding facility at the University of Tel Aviv, and were raised in the pathogen free animal facility at the Faculty of Medicine, Technion, Haifa.

Skin grafting

In these experiments, 2 mm punch grafts obtained from remnants of scalp skin of normal individuals undergoing elective face lift procedures were used for transplantation.

The skin was unshaved prior to transplantation in order to observe growth and shedding of hairs. Each 2 mm graft was inserted through a small incision in the skin into the subcutaneous tissue over the lateral thoracic cage of a mouse and covered with a standard band aid dressing. The dressings were removed on day 10 and thereafter the number of hairs in each graft was counted and the length of a sample of hairs measured under magnification twice weekly. Each count was made by two different individuals. In total, 25 mice were engrafted and commenced on twice daily topical treatment on day 10. Ten mice, designated Group A, were treated with topical CyA (cyclosporin, 50 mg/ml, Sandoz Laboratories, East Hanover, NJ, U.S.A.) in olive oil. Each application consisted of 1 mg CyA in 0·02 ml olive oil. Fifteen mice were treated initially with olive oil. One week later (day 17) five mice in the olive oil group, designated Group B, were switched to CyA treatment. The remaining 10 mice, constituting the control group, were continued on olive oil treatment. Standard adhesive gauze was used to prevent the animals from licking the grafts. The animals were treated until day 38 following engraftment.

TABLE 1. Topical treatment with cyclosporin on grafted skin. Parameters of hair growth

	Day	No. of grafts with hair (%)	No. of hairs per graft (mean ± SD)
Group A	10	19/21 (91)	4·4 ± 1·8
(CyA from day 10)	17	20/21 (95)	3·7 ± 1·4
	24	18/21 (86)*	3·2 ± 1·2**
	31	11/21 (52)*	2·7 ± 1·1**
	38	10/21 (48)*	1·8 ± 0·4**
Group B	10	8/10 (80)	3·6 ± 1·9
(CyA from day 17)	17	5/10 (50)	2·8 ± 1·5
	24	4/10 (40)	2·7 ± 1·0
	31	4/10 (50)	2·0 ± 0·7
	38	4/10 (40)	1·7 ± 0·5
Control	10	14/16 (88)	4·1 ± 1·9
(olive oil only)	17	6/16 (38)	3·2 ± 1·9
	24	0·16 (0)	0
	31	0/16 (0)	0
	38	1/16 (6)	1·0

* Significantly different from control group ($P < 0{\cdot}005$; chi-square test).

** Signifficantly different from control group ($P < 0{\cdot}01$; Student's *t*-test).

At the end of the study (day 38) the engrafted skin was examined histologically. Statistical analysis was carried out using Student's *t*-test and Chi-square tests.

RESULTS

The hair growth results in the three groups are summarized in Table 1. In the group treated with CyA from day 10 (Group A) the number of grafts with hair remained nearly constant until day 24 (18 out of 21, (86%)), showing thereafter a gradual reduction in the number of grafts with hair (10 out of 12 (48%)), and the number of hairs per graft. Many of the originally grafted hairs were shed and new hairs appeared between days 31 and 38. By day 38 10 of 21 grafts in this group had hair. Of these 10 grafts, four contained only new hairs (data not shown).

More obvious shedding was demonstrated in the control group. A decrease in the number of grafts with hair was noted by day 17 and complete shedding by day 24. The shedding in the CyA treated group (Group A) was significantly less than in the control group ($P < 0{\cdot}005$). A significant difference was also found in the mean number of hairs per graft ($P < 0{\cdot}01$).

The results in Group B, treated with CyA only after 17 days resembled those in Group A, namely the process of shedding was slowed down from day 24 onwards as measured by both numbers of grafts with hair and numbers of hairs per graft.

Histological preparations of engrafted skin from the groups were examined and compared. In the CyA treated group (Group A) the histological findings confirmed the clinical observation of an increased hair count. Hair follicles in the anagen phase were observed (1–2 per graft) in Group A while no follicles in this phase were seen in the control group. The hair shafts had a mean diameter (±SD) of $0{\cdot}04 \pm 0{\cdot}01$ mm, while the diameter in the initial explant was $0{\cdot}06 \pm 0{\cdot}01$. No other differences in morphology between the three groups were observed.

DISCUSSION

Considerable evidence now exists that CyA has an effect on hair growth. Hypertrichosis occurs in up to 40% of patients treated with systemic CyA for various conditions.[2] The exact mechanism by which hypertrichosis develops is far from clear and its pathogenesis is still obscure.[2] Oral administration of CyA promotes hair growth in mouse follicles.[5] CyA has been proposed as a potential therapy for several skin disorders, mainly those with an autoimmune aetiology.[6] Alopecia areata and alopecia universalis are thought to be immune mediated, and we have previously shown the beneficial effect of systemic CyA in nude mice grafted with skin from human alopecia areata and alopecia universalis. However, the side-effects of systemic CyA therapy are numerous and nephrotoxicity is a particular problem.[6] It was important, therefore, to determine whether the beneficial effect of systemic CyA[7] was also produced on topical application. No side-effects have been observed to date with topical CyA.[4] Furthermore, we wanted to determine whether the same effect would be produced in normal skin grafts which had not been subjected to immunological damage.

Topical CyA administration significantly retarded the immediate shedding of hairs following skin transplantation. In the group treated with CyA throughout (Group A) the number of grafts with hairs remained nearly constant until day 24, in contrast to the control group in which a reduction was noticed by day 17 and complete shedding by day 24. The rapidity of shedding in our model (similar to that seen in hair transplantation) is unlike normal telogen transformation and is more suggestive of an anagen effluvium, which may be significantly reduced by CyA. The persisting decrease in the number of hairs found in the present study may, alternatively, reflect increasing passage of follicles from the anagen phase into the catagen and telogen phases. Therefore, our observation may indicate that CyA slows down this transition by prolonging the anagen phase. It is clear that more histological data is needed to confirm this possibility. Moreover, the tendency to appearance of new hairs suggests that CyA may also accelerate the onset of anagen in the results.

It should be noted that no hypertrophy of follicles was observed on the basis of the diameter of the hairs as well as their morphology.

Further studies are needed to elucidate in more detail the exact mechanism of action of CyA on hair shedding and growth.

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The effects of topically applied hexyl nicotinate lotion on the cutaneous blood flow in patients with Raynaud's phenomenon

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SUMMARY

The effect of the epicutaneous application of 0·1% and 1·0% hexyl nicotinate lotion on upper limb skin blood flow was investigated in 25 patients with Raynaud's phenomenon (RP) and five healthy volunteers. Assessments were made using laser Doppler velocimetry.

The 0·1% lotion induced an increase in cutaneous blood flow in both controls and patients when applied at different sites on the upper limb. Increased cutaneous blood flow occurred more often when the lotion was applied to the forearm than to the fingers. A greater increase in blood flow and an increased percentage of positive responses was elicited with the 1·0% hexyl nicotinate lotion. Hexyl nicotinate lotion may be of value in the topical treatment of RP.

Raynaud's phenomenon (RP) remains a difficult therapeutic problem despite recent advances using new vasodilators such as prostaglandins[1,2] and calcium channel blocking agents.[3–5] Treatment with prostaglandins requires hospital-based administration of intravenous infusions, and side-effects such as headache, flushing and hypotension commonly occur during treatment with both calcium channel blocking agents and prostaglandins. Epicutaneous administration of glyceryl trinitrate[6] and a prostaglandin E_2 analogue[7] have proved of some benefit, but systemic side-effects are also encountered.

Several nicotinic acid esters induce vasodilation after epicutaneous application and it has been suggested that they may be effective vasodilators by virtue of their ability to increase local synthesis of prostaglandins.[8,9] Furthermore, a favourable response to systemic inositol nicotinate, which is metabolized to nicotinic acid *in vivo*, has been reported in RP.[10] We have previously demonstrated hexyl nicotinate lotion to be an effective cutaneous vasodilator at several anatomical sites.[11,12] We, therefore, decided to investigate its effects on upper limb cutaneous blood flow in patients with Raynaud's phenomenon. We employed laser Doppler velocimetry to measure the changes in cutaneous blood flow.[13]

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METHODS

Twenty five patients took part in the study 13 with primary Raynaud's disease (*primary RP*) (12 females, one male, mean age 35 years, range 24–55) and 12 with systemic sclerosis and RP (11 females, one male, mean age 47 years, range 21–62). The diagnosis of primary or secondary RP was established from the history, physical examination, and appropriate laboratory investigations to detect the presence of an underlying cause for secondary RP. These investigations routinely comprised full blood count, ESR, urea and electrolytes, liver function tests, creatinine clearance, autoantibody screen including anticentromere and anti-Scl 70 antibodies, extractable nuclear antigen (ENA), cryoglobulins, protein electrophoresis, circulating immune complexes, chest X-ray, barium swallow in the Trendelenberg position, meal and follow-through, respiratory function tests and ECG. Five healthy controls, mean age 31 years (range 21–39) were also included.

Hexyl nicotinate (HN), 0·1% and 1%, in a lotion formulation containing zinc stearate 1%, stearic acid 5%, cetosterol alcohol 4%, cetrimide 1%, dimethicone 350 7·5%, isopropyl myristate 7·5%, glycerol 25%, chlorocresol 0·1% and water to 100% were supplied by Dermal Laboratories, Hitchin, Herts, U.K. HN lotion, 0·025ml, was simultaneously applied to 2cm^2 diameter symmetrical test sites on the skin of the ventral surface of the forearm, the dorsal surface of the hand or the dorsal surface of the middle phalanx of the fingers of both hands. When it became apparent that an increase in blood flow was invariable at the proximal forearm sites, attention was focussed on examining the responses at the more distal sites, i.e. the fingers, where an increase in blood flow was less readily elicited. Vehicle alone was similarly applied to separate forearm, hand and digital sites in some subjects as a control.

All assessments took place in the Vascular Laboratory of the Middlesex Hospital at an ambient temperature of 19–22°C. Assessments were started after 30 min acclimation in this environment with the subject seated and the arms supported at the level of the heart. No patient had had a hot or alcoholic drink within 3h of the assessment, nor had they smoked on the day of assessment and they were taking no systemic medications.

Erythema was observed at sites on one upper limb and simultaneous recordings of cutaneous blood flow were made at the corresponding contralateral site. The cutaneous blood flow was assessed using laser Doppler velocimetry (PeriFlux, Perimed, Sweden). Laser light is transmitted to the skin by an optical fibre and penetrates to a depth of 1mm. Light is backscattered from stationary skin structures and by red blood cells moving in the dermal microvasculature. This is detected by efferent optical fibres. The instrument separates out the Doppler shifted component, due to the moving elements and converts it into a flux parameter, which correlates with cutaneous blood flow. A voltage signal is generated which enables a continuous graphical read-out to be obtained on a chart recorder. The increase in blood flow was measured as the maximal increase in the flux value during the monitoring period. Baseline blood flow readings were made at the selected site for 5 min prior to the application of the lotion. The probe was then replaced on the skin at precisely its original site and recording continued until the blood flow had returned to baseline, or for 30 min, if no change in blood flow occurred. A positive response to the lotion was taken as a greater than 1·5-fold increase in flux from baseline. The times to maximum increase and return to baseline were recorded. The difference in response at the finger sites to the two strengths of the HN lotion was analysed using a chi-squared test.

RESULTS

There was no difference in baseline blood flow between the two patient groups and the controls. There was no response at any site in any patient or control to application of the vehicle alone.

Responses of controls to 0·1% hexyl nicotinate lotion

The responses of the five normal controls to the 0·1% lotion are shown in Table 1. Seventeen sites were assessed. It can be seen that there was a 100% response to application of the lotion to the forearm with a median increase in flow of 6·7-fold (range 5·0–31·0) and this was accompanied by erythema in each case. There was only one response out of three applications to the hands and only one response out of nine applications to the fingers, with no erythema. The time to maximal effect after applying the lotion ranged from 9–17 min and in all cases this persisted for 30 min or more.

TABLE 1. Responses in five normal controls to 0·1% hexyl nicotinate lotion

Site	No. of sites tested	No. of responses (%)	Increase in flow measured as flux (mV) (median and range)	No. of sites showing erythema (%)
Forearm	5		6·7(5·0–31·0)	4(80)
Hand	3	1(33)	4	0
Fingers	9	1(11)	3	0

Responses in patients with primary Raynaud's phenomenon to 0·1% hexyl nicotinate lotion

Forty sites were assessed in 12 patients with primary RP and the results are shown in Table 2. As in the normal controls there was a 100% response at the sites of application on the forearms, and all these sites showed erythema. There was a median increase in blood flow of eightfold (range 5·0–15·0) over baseline. Application to the hands resulted in increase in blood flow at five of 12 (42%) of the sites assessed, with a median increase in flow of 7·6-fold (range 3–10). Erythema was visible at only three of the contralateral sites and only occurred in subjects who exhibited an increase in blood flow. A twofold increase in blood flow occurred at only three out of 25 sites to which lotion was applied on the fingers. Erythema was only visible at one site in one of these patients. The time to maximal effect of the lotion ranged from 8–38 min and this persisted for over 60 min in seven of the 11 positive responses.

TABLE 2. Responses in 12 patients with primary RP to 0·1% hexyl nicotinate lotion

Site	No. of sites tested	No. of responses (%)	Increase in flow measured as flux (mV) (median and range)	No. of sites showing erythema (%)
Forearm	3	3(100)	8·0(5·0–15·0)	3(100)
Hand	12	5(42)	9·0(3·0–10·0)	3(25)
Fingers	25	3(12)	2·0	1(4)

Responses in patients with secondary Raynaud's phenomenon to 0·1% hexyl nicotinate lotion

Thirty five sites were assessed in 11 patients with systemic sclerosis and RP. The results are shown in Table 3. There was a 100% response to the lotion on the forearm, but only one of the three patients showed contralateral erythema. There was a threefold median increase in blood flow (range 3·0–6·5). When the hand sites were tested, six out of 11 patients (55%) responded with a median increase in blood flow of fourfold (range 1·6–30·0) and two of the responders developed erythema at the contralateral test site. Seven out of 21 (33%) applications of the 0·1% HN lotion to the fingers elicited an increase in skin blood flow; the median increase was twofold (range 1·8–3·0). No responder developed erythema. The time to maximal effect of the lotion after application was between 1 and 36 min and this persisted for over 30 min in every case and over 60 min in four cases.

TABLE 3. Responses in 11 patients with secondary RP to 0·1% hexyl nicotinate lotion

Site	No. of sites tested	No. of responses (%)	Increase in flow measured as flux (mV) (median and range)	No. of sites showing erythema (%)
Forearm	3	3(100)	3·0(3·0–6·5)	1(33)
Hand	11	6(55)	4·0(1·6–30)	2(18)
Fingers	21	7(33)	2·0(1·8–3·0)	0

TABLE 4. Overall responses of 23 patients with Raynaud's phenomenon to 0·1% hexyl nicotinate lotion

Site	No. of sites tested	No. of responses (%)	Increase in flow measured as flux (mV) (median and range)	No. of sites showing erythema (%)
Forearm	6	6(100)	5·7(3·0–15·0)	6(100)
Hand	23	11(48)	7·5(1·6–30·0)	5(21)
Fingers	46	10(22)	2·0(1·8–3·0)	1(2)

Overall responses to 0·1% hexyl nicotinate lotion

The overall response rate for all patients with RP, both primary and secondary, are shown in Table 4.

Responses in primary and secondary RP to 1% hexyl nicotinate lotion

Seven patients with RP, 4 primary and 3 secondary, were tested at 13 digital sites with 1% HN lotion. Five out of the seven (71%) responded at seven of the 13 sites (54%). This is a statistically significant improvement in response compared with that to 0·1% HN lotion ($P < 0{\cdot}01$). Only one responder (8%) developed erythema at a contralateral test site. The median increase in blood flow was 4·2-fold (range 2·6–8·5) and the time to maximal effect ranged between 16 and 46 min. The effect persisted for between 27 and 78 min.

There were no side-effects from application of the lotion apart from a feeling of warmth at the site of application in two patients, which was not considered unpleasant. Concurrent measurements of skin blood flow at sites away from the test site showed that the effect of the lotion was localized to the site of application. It was noticed that the act of rubbing the lotion into the skin produced an immediate increase in blood flow in some patients. This was in the range of 1·5–5·0 fold and persisted for up to 10 min before returning to baseline and preceded the effects elicited by the HN lotion. There was no case of a positive response to rubbing at 12 sites tested in the controls.

DISCUSSION

The cause of RP is unknown, but abnormalities of total hand or finger blood flow[14] and of capillary blood flow[15] have been demonstrated in patients with RP. Total finger and cutaneous blood flow can be increased in patients with RP by vasoactive drugs which can produce clinical benefit in RP[16].

Using laser Doppler velocimetry we have demonstrated that the epicutaneous application of hexyl nicotinate lotion increased skin blood flow in both normal individuals and patients with RP.

An increase in cutaneous blood flow occurred at every application of the 0·1% lotion to the forearm, but responses were attenuated at more distal application sites. In responders, no patient responded at all sites to this strength of lotion. With the 1% HN lotion the response rate was increased at the distal, digital sites.

When the lotion was applied to the dorsum of the fingers the response rate was increased from 22% to 54% by increasing the concentration of the HN from 0·1% to 1%.

Increased flow rates occurred without discomfort or erythema and persisted for 30 to 60 min. The failure of some RP patients to show erythema while exhibiting increased cutaneous blood flow may be because the superficial vessels are unable to dilate because of skin stiffness. It may be the dilatation of deeper blood vessels which contributes to the increased blood flow.

The present study has shown that the percutaneous application of a nicotinic acid ester can produce a substantial localized increase in cutaneous blood flow in a proportion of patients with primary and secondary RP without significant side-effects. This may be of therapeutic value in patients suffering from RP who are intolerant of systemic vasodilators. A controlled trial of the efficacy of hexyl nicotinate in the treatment of RP is currently in progress.

Although we found erythema at the site of application to be an unusual and asymptomatic occurrence, inadvertent transfer of the lotion to other sites for example, the face may occur and patients should be warned about this. We have no information concerning blood flow around a site of application. It is conceivable that there is a 'steal' phenomenon and we do not recommend the use of the lotion around ulcerated skin.

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X-linked ichthyosis and ichthyosis vulgaris: comparison of their clinical features based on biochemical analysis

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SUMMARY

Thirty cases of X-linked ichthyosis (XLI) and 32 cases of ichthyosis vulgaris (IV) were diagnosed by measuring the steroid sulphatase activity of peripheral blood lymphocytes or the electrophoretic mobility of serum LDL or both. The clinical features of the two conditions were then compared. In both diseases 60–66% of patients had a family history of the condition. Ichthyosis was noted at birth or immediately afterwards in 59% of the patients with XLI while it appeared in infancy in 68% of those with IV. Scales were mostly large and brown or dark brown in patients with XLI, while the majority of patients with IV had small brown or light brown scales. The distribution of the ichthyotic lesions differed in the two types of ichthyosis. On the trunk, the abdomen was more severely involved than the back in 63% of the cases with the XLI, whereas the back was more scaly than the abdomen in 44% of those with IV. On the extremities, the extensor surface was more severely affected than the flexor surface in both types. X-linked ichthyosis was characterized by the presence of lesions in the pre-auricular area, which were found in 93% of the cases with XLI, while only 17% of the IV patients had ichthyotic lesions at this site. Involvement of the preauricular area could be an important clinical feature for distinguishing XLI from IV.

In 1933, Cockayne[1] described a sex-linked form of ichthyosis which resembled ichthyosis vulgaris (IV), but occurred in males only—X-linked ichthyosis (XLI). The classical diagnostic criteria for XLI in contrast to those for IV are as follows.[2–7] In typical XLI, the mode of inheritance is sex-linked recessive and so only males are affected; the ichthyosis is noted at birth or immediately afterwards and diagnostic criteria for collodion baby are sometimes found at about this time; the scales tend to be large and dark, 50% of cases have ichthyosis on the pre-

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auricular area, and the abdomen is more severely involved than the back in three-quarters of patients; dry scaling is evident on both extensor and flexor surfaces of the limbs, more than one-third of patients show ichthyotic changes in the flexures of the limbs, while the palms and the soles are clinically normal; histopathologically, the granular layer is conspicuous or normal; ophthalmologically, corneal opacity is frequently present.

Clinical distinction between XLI and IV, however, is actually difficult in many cases, because the disorders essentially resemble each other in their cutaneous manifestations. However, Shapiro *et al.*[8] demonstrated that XLI is deficient in steroid sulphatase activity, while Epstein *et al.*[9] reported that the electrophoretic mobility of low-density lipoprotein (LDL) is greatly elevated owing to accumulated plasma cholesterol sulphate in XLI.[10] We recently reported that polyacrylamide gel disc electrophoresis is useful for the detection of electrophoretic mobility of LDL.[11] These studies not only form the basis for clarification of the aetiology of XLI,[12–15] but also have made it possible to clearly distinguish XLI from IV biochemically, a distinction which is not easy to make clinically. The present study was undertaken to clarify the clinical features of XLI and IV diagnosed by determination of the steroid sulphatase activity of peripheral lymphocytes or the electrophoretic mobility of serum LDL or both.

METHODS

Subjects

We studied 30 cases with XLI (all male, aged 10 months–75 years; mean age 27 years) and 32 cases with IV (24 male, eight female; aged 1–75 years, mean 32 years). Patients in both groups were diagnosed by measuring the steroid sulphatase activity of peripheral blood lymphocytes or the electrophoretic mobility of serum LDL or both.

Assay of steroid sulphatase activity and electrophoretic mobility of serum LDL

Steroid sulphatase activity of lymphocytes was measured using 7-[^{3}H] (N) dehydroepiandrosteronesulphate (40–90 Ci/mol) as a substrate, according to the method of Epstein *et al.*,[16] modified by Okano *et al.*[17,18] Serum LDL was subjected to disc electrophoresis in 3% polyacrylamide gel; the measurement of the relative electrophoretic mobility of LDL has also been described elsewhere.[17]

Clinical features

In both types of ichthyosis, we examined the correctness of the clinical diagnosis (based on clinical examination by medical staff in our clinic and other dermatology clinics in the Osaka area, who were blind to the biochemical data). We also compared family history and age at onset (obtained from patient questionnaires), cutaneous manifestations (in the cold season), histopathological features and ophthalmological findings (corneal opacity). The last three assessments were made by the authors.

RESULTS

The features of the patients with XLI are listed in Table 1, the features of patients with IV in Table 2, and the two conditions are compared in Table 3.

Clinical features of X-linked ichthyosis

Clinical diagnosis and family history. Nineteen (63%) of 30 cases with XLI were identified as

TABLE 1. Clinical and biochemical features of 30 cases of X-linked ichthyosis

Case no.	Family history	Time of onset	Clinical diagnosis	Histological features	Corneal opacity	Steroid-sulphatase (pmol DHEA/mg protein/h)*	Elevated electrophoretic mobility of LDL
1	+	Birth	IV	IV	+	0	+
2	+	Infancy	XLI	...	+	0	+
3	+	1 Year	XLI	...	−	0·4	+
4	+	Birth	XLI	...	...	0	...
5	+	Birth	XLI	XLI	−	0	+
6	+	Birth	XLI	...	...	8·6	+
7	+	Birth	XLI	...	...	2·8	+
8	+	Birth	XLI	XLI	...	0	+
9	+	Birth	XLI	...	...	0	...
10	−	Birth	IV	...	+	0·1	+
11	+	6 Months	XLI	...	...	0·1	...
12	−	Infancy	XLI	XLI		10·5	+
13	+	1 Year	XLI	...	+	5·2	...
14	−	Infancy	IV	IV	+	9·6	+
15	−	1 Year	IV	XLI	−	2·8	+
16	−	Birth	IV	IV	...	0	...
17	−	Infancy	IV	...	...	0	...
18	+	Birth	XLI	XLI	−	0	+
19	+	Birth	IV	IV	...	0	...
20	+	6 Months	XLI	XLI	−	3·0	+
21	−	Birth	IV	IV		3·4	...
22	+	Birth	XLI	...	−	...	+
23	+	Birth	XLI	...	−	...	+
24	+	Infancy	XLI	...	+	...	+
25	−	6 Months	XLI	...	...	...	+
26	+	Birth	XLI	...	+	...	+
27	−	Birth	IV		...	0	...
28	+	7 Years	IV	...	+	0	+
29	−	Birth	XLI	...	...	...	+
30	−	5 Years	IV	...	−	...	+

XLI = X-linked ichthyosis; IV = Ichthyosis vulgaris; *Normal controls (male): 122·2 ± 19·3.

having clinically ichthyotic males in their family pedigrees. Sixteen (84%) of these 19 cases were correctly diagnosed as XLI on the basis of clinical features and family history. Of the 11 cases without any ichthyotic relatives, only three cases (27%) were clinically diagnosed as having XLI.

Age at onset. Seventeen (59%) of the 29 cases examined had clinical ichthyosis at birth and 10 (34%) before the age of one year.

Histopathology of the skin. A granular layer was present in six (55%) of the 11 cases examined and was thin in five (45%).

Ophthalmological findings. Deep stromal opacity of the cornea was detected by slit-lamp examination in nine (50%) of the 18 cases examined.

TABLE 2. Clinical and biochemical features of 32 cases of ichthyosis vulgaris

Case no.	Sex	Family history	Time of onset	Clinical diagnosis	Histological features	Corneal opacity	Steroid sulphatase (pmol DHEA/mg protein/h)	Elevated electrophoretic mobility of LDL
1	M	+	Infancy	IV	...	...	126·6	...
2	M	–	3 Months	XLI	...	–	140·6	–
3	F	+	Birth	IV	...	...	212·0	–
4	M	+	12 Years	IV	IV	...	97·4	–
5	M	+	4 Years	XLI	IV	...	123·4	–
6	M	+	Birth	IV	IV	...	142·9	...
7	M	+	Infancy	IV	...	...	193·2	...
8	M	+	6 Years	XLI	...	–	125·6	...
9	F	–	13 Years	IV	...	–	244·9	...
10	M	+	Birth	IV	IV	...	129·4	...
11	F	–	6 Months	IV	...		266·5	
12	M	+	5 Years	IV	...	...	101·3	...
13	F	+	7 Years	IV	...	...	237·9	...
14	M	+	7 Years	IV	...	–	175·2	...
15	M		20 Years	XLI	IV	...	112·8	–
16	M	+	8 Years	IV	IV	–	118·4	...
17	M	–	50 Years	IV	...	–	164·8	...
18	M	–	1 Year	XLI	...	–	141·3	...
19	M	+	1 Week	IV	...	–	109·8	...
20	F	+	Birth	IV	...	–	150·0	...
21	M	+	Birth	XLI	...	...	125·6	...
22	M	+	Birth	IV	...	...	105·2	...
23	F	+	7 Years	IV	...	–	154·2	...
24	M	–	1 Month	IV	...	...	189·4	...
25	M	–	30 Years	IV	IV	...	132·7	...
26	F	+	1 Year	IV	...	–	227·0	...
27	F	+	6 Months	IV	...	–	151·2	...
28	M	+	1 Year	XLI	...	–	...	–
29	F	+	3 Year	IV	...	–	...	–
30	M	–	7 Year	IV	...	–	...	–
31	M	–	Infancy	XLI	...	...	...	–
32	M	–	Infancy	XLI	...	...	...	–

XLI: X-linked ichthyosis; IV: Ichthyosis vulgaris.
Normal controls ♂: 122·2 ± 19·3
♀: 187·8 ± 21·0.

Size and colour of the ichthyotic scales. Twenty-one (81%) of the 26 cases examined had large scales, and the rest (19%) had small scales. The scales were dark brown in 14 cases (54%) and brown in 12 (46%). There were no cases with light-brown or white scales.

Distribution of the lesions. The trunk: The abdomen was more severely affected than the back in 19 (63%) of the 30 cases, the back more severely involved than the abdomen in three (10%) and both were affected to a similar degree in eight (27%).

The extremities: Of the 29 cases examined, the extensor surface was more severely involved

TABLE 3. Comparison of clinical features of X-linked ichthyosis (XLI) and ichthyosis vulgaris (IV)

Type of scale

Size	Large	Small
XLI	21 (81%)	5 (19%)
IV	11 (34%)	21 (66%)

Colour	Dark brown	Brown	Light brown
XLI	14 (54%)	12 (46%)	0 (0%)
IV	5 (16%)	12 (38%)	15 (46%)

Distribution of scales

Trunk	Abdomen > back	Abdomen = back	Abdomen < back
XLI	19 (63%)	8 (27%)	3 (10%)
IV	10 (31%)	8 (25%)	14 (44%)

Extremities	Flexor > extensor	Flexor = extensor	Flexor < extensor
XLI	4 (14%)	4 (14%)	21 (72%)
IV	3 (9%)	1 (3%)	28 (88%)

		Scales present	Scales absent
Preauricular area	XLI	25 (93%)	2 (7%)
	IV	5 (17%)	25 (83%)
Flexures	XLI	12 (41%)	17 (59%)
	IV	10 (31%)	22 (69%)
Palm and sole	XLI	3 (11%)	24 (89%)
	IV	12 (39%)	19 (61%)

than the flexor in 21 (72%), and the reverse was true in four (14%), while both were involved to a similar extent in the remainder (14%).

The pre-auricular area: Ichthyotic lesions were found in 25 (93%) of the 27 cases examined.

The flexures: Of a total of 29 cases examined, 12 (41%) had ichthyotic scales in the flexures.

The palms and soles: Lesions were seen on the palms and soles in three (11%) of the 27 cases examined.

Clinical features of ichthyosis vularis

Clinical diagnosis and family history. Of a total of 32 cases with IV, 21 (66%) had ichthyotic relatives. Of these 21, 17 (81%) were correctly diagnosed as IV on the basis of clinical features and family history. Clinical diagnosis of IV was established in 45% of the 11 cases without any family history of ichthyosis.

Age at onset. Twenty-two (68%) of the 32 cases had ichthyotic lesions in early infancy, six (19%) at birth, and four (13%) in early adulthood.

Histopathology of the skin. All of the seven biopsy specimens examined showed a thin or absent granular layer.

Ophthalmological findings. Slit-lamp examination did not detect deep corneal opacity in any of the 17 cases examined.

Size and colour of ichthyotic scales. Of the 32 cases, 11 (34%) had large scales, and the rest (66%) had small scales. The colour of the scales was dark brown in five cases (16%), brown in 12 (38%) and light brown in the remainder (46%).

Distribution of the lesions. The trunk: The abdomen was more severely involved than the back in 10 (31%) of the 32 cases, the back more severely affected than the abdomen in 14 (44%), and both were involved to a similar degree in the remainder (25%).

The extremities: Of the 32 cases, the extensor surface had more severe ichthyotic scaling than the flexor in 28 cases (88%), the flexor surface had more severe lesions than the extensor surface in three (9%), and both were affected to a similar degree in one case (3%).

The pre-auricular area: Ichthyotic scales were seen in the pre-auricular area in five (17%) of the 30 cases examined.

The flexures: Of the 32 cases, 10 (31%) had ichthyotic lesions in the flexures.

Palms and soles: Scales were found on the palms and soles in 12 (39%) of the 31 cases examined.

DISCUSSION

In our study, based on biochemical analysis, more than 80% of the XLI cases who had ichthyotic relatives were clinically diagnosed as XLI. In contrast, a clinical diagnosis of IV was made in most of the sporadic XLI cases, although half of the sporadic IV cases were clinically diagnosed as having IV. Accordingly, making a correct clinical diagnosis seems difficult in sporadic XLI cases because of close resemblance of the clinical features of the two types of ichthyosis. On histological examination, 56% of the XLI specimens showed the conspicuous or normal granular layer which Wells *et al.*[5] considered characteristic of the disorder. The granular layer was thin or absent in all of the IV specimens, as reported by Wells *et al.*[5] In contrast to the report by Sever *et al.*,[7] only half of our patients with XLI had corneal opacity, whereas none of those with IV did. Our data on ichthyotic scales, by and large confirmed the reports by Wells *et al.*[4,5,7] and Kerr *et al.*[3] on cutaneous manifestations, including colour and size of the scales and their distribution. The extensor surface of the extremities, however, was more severely affected in our cases of XLI as well as in those of IV. Even the palm or the sole was involved in 11% of our XLI cases. Surprisingly, not half of the cases, as reported by Wells *et al.*,[2,4–6] but almost all of our XLI cases had ichthyotic scales in the preauricular area, whereas pre-auricular involvement was seen in only 17% of the IV cases. Clinically, therefore, XLI may be characterized by pre-auricular involvement, and this seems to be an important clinical feature for distinguishing XLI from IV.

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Metastatic calcification and cytomegalovirus infection

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SUMMARY

We report a patient who developed hypercalcaemic metastatic cutaneous calcification in association with a cytomegalovirus infection after a liver transplant. The skin nodules disappeared as the hypercalcaemia resolved and the cytomegalovirus infection subsided.

Metastatic cutaneous calcification is an uncommon complication of hypercalcaemia associated with hyperparathyroidism, sarcoidosis, vitamin D excess, milk-alkali syndrome and destructive bone disease.[1] We report here the first case, to our knowledge, in association with cytomegalovirus infection.

CASE REPORT

A 23-year-old Caucasian women had a partial hepatectomy for a fibrolamellar hepatoma in 1984. A liver transplant was performed in 1986 for local tumour recurrence. Post-operatively she required assisted ventilation and haemodialysis for acute renal failure, but within a month she was off dialysis and the transplanted liver was functioning satisfactorily although she continued to have a low-grade pyrexia and malaise. In mid-December 1986 subcutaneous nodules began to erupt (Fig. 1). They were noted initially over her sternum and left shin, but within 10 days they had developed in most areas of her body. The nodules were mildly painful, but not inflammed. They varied in size from 1 to 4 cm and were remarkable for their hardness. A skin biopsy showed dense deposits of calcium in the deep dermis and subcutis (Fig. 2), confirmed by staining with alizarin red and the method of Von Kossa. There was little inflammatory response and no evidence of osteoclasts or tumour. A technetium^{99} bone scan showed a normal pattern of uptake in the skeleton with soft tissue hot spots corresponding to the cutaneous nodules (Fig. 3).

The post-transplant serum calcium and albumin-corrected calcium levels are shown in

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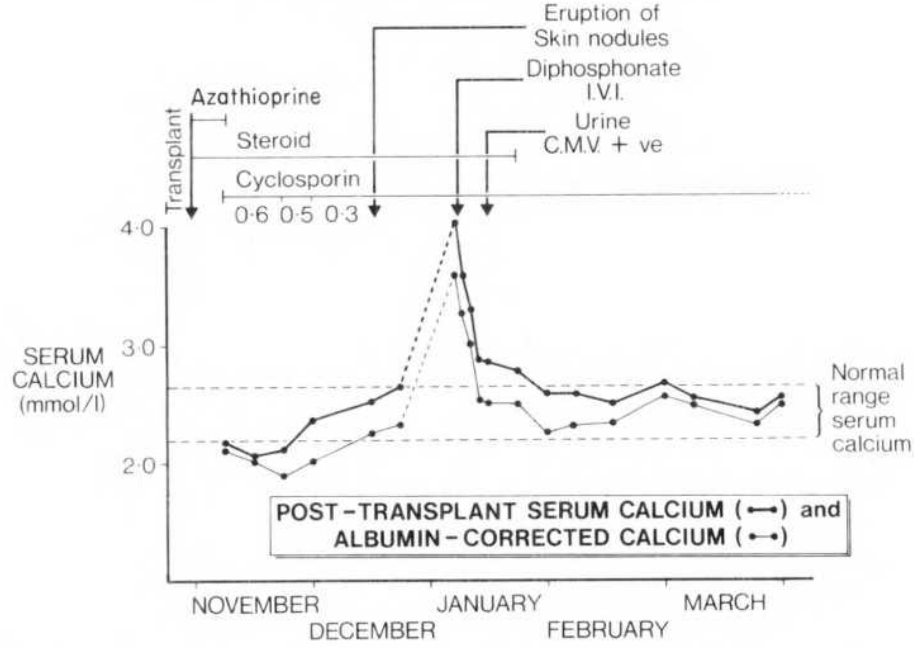

FIGURE 1. Serum calcium levels, immunosuppressive therapy and progress following liver transplant. Dotted lines indicate Christmas break.

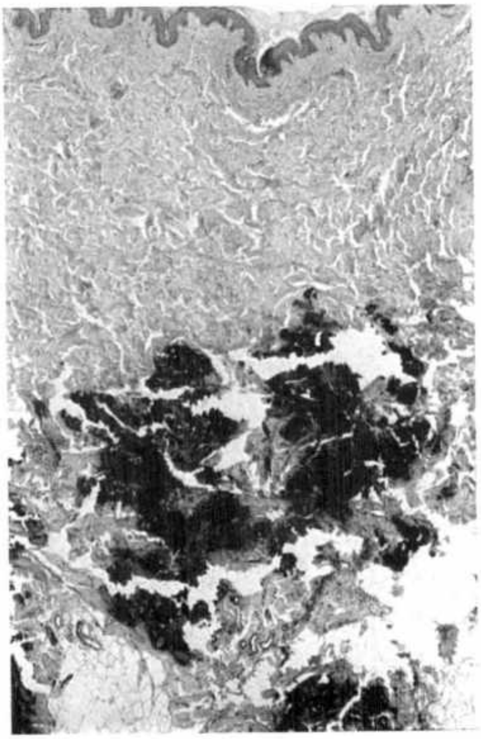

FIGURE 2. Deposits of calcium in the deep dermis and fat. Haematoxylin & Eosin × 115.

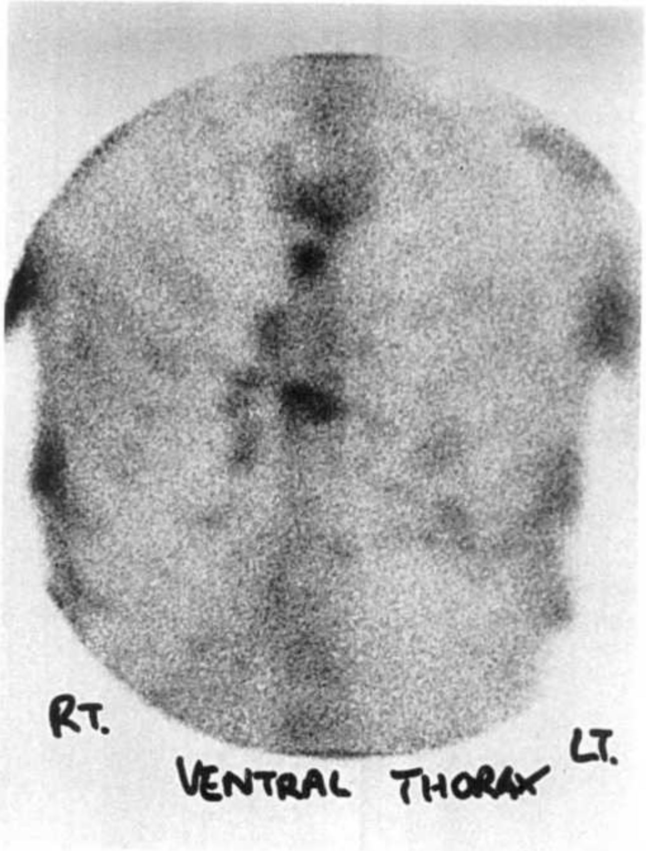

FIGURE 3. Parasternal uptake of Tc^{99} on ventral thoracic isotope scan.

Figure 1. Although the calcium level was rising when the nodules erupted it remained within the normal range until after the patient had returned from a Christmas break in Scotland. There was a rapid fall following rehydration and an infusion of diphosphonate. One week later cytomegalovirus was isolated from her urine on human embryo lung cultures. On discontinuation of steroid therapy the pyrexia resolved and urinary excretion of the virus ceased within 2 weeks. By the end of January 1987 the serum calcium level had returned to normal and the skin nodules had disappeared. At the peak of the hypercalcaemia, serum parathormone levels were undetectable by radioimmunoassay, serum phosphate was at the upper limit of the normal range and alkaline phosphatase was considerably increased at 4500 U/l (isoenzymes showed that this was all hepatic in origin). Urea and creatinine remained stable and within the normal ranges from Christmas onwards. She remained well at the time of writing, although two post-transplant liver biopsies both showed evidence of chronic rejection with loss of bile ducts.

DISCUSSION

It seems most likely that the hypercalcaemia was due to acute cytomegalovirus infection contracted from a blood transfusion, as pre-operative screening of the liver donor and recipient were negative. Hypercalcaemia has been reported in two patients with the acquired immune deficiency syndrome and cytomegalovirus infection,[2] but the mechanism is unknown. The authors speculated that direct stimulation of osteoclasts by the virus or the production of a parathormone-like factor, not detectable on routine assays, may have occurred.

Although chronic renal failure and malignancy are recognized causes of hypercalcaemia, neither are likely to account for this case as renal function had returned to normal before the serum calcium began to rise. The hepatic tumour was completely resected 6 weeks before the nodules erupted. The possibility that the hypercalcaemia may have been related to cyclosporin therapy is also unlikely as the daily dose remained unchanged at 0·3 g before and after its development.

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Linear IgA dermatosis with severe arthralgia

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SUMMARY

Two patients aged 8 and 34 years, with linear IgA dermatosis and systemic symptoms are described. Both presented with fever and sore throat 5 to 10 days before the onset of their rash and both developed severe arthralgia accompanying the skin eruption. The joint pains resolved when the skin eruption was treated.

CASE REPORTS

Patient 1

An 8-year-old farmer's son presented with a sore throat, fever and abdominal pain followed 5 days later by myalgia and arthralgia. Three days after the onset of the joint pains he developed a vesicular eruption on the nose. He was treated with topical acyclovir and erythromycin, but continued to have sharp pains in the muscles of his forearms and calves, and in the joints of his ankles, wrists and elbows. The joints were not swollen at any time. Aspirin provided some relief. There was associated mild headache and an intermittent pyrexia.

His joint symptoms persisted and he developed a low grade progressive anaemia over the next 2 months. At this time the spleen tip was noted to be palpable. He was found to have tonsillitis which was treated with a course of penicillin in high dosage.

By the 11th week he had developed vesicles on his arms, neck and ears in addition to those on his face. These were thin-roofed, friable, mildly pruritic and grouped in an herpetiform fashion. The joint pains persisted.

In the 17th week he developed the 'cluster of jewels' sign, characterized by a circle of new lesions surrounding a central healing area (Fig. 1). At this time his parents finally agreed to skin biopsies being carried out. The diagnosis of linear IgA dermatosis (bullous dermatosis of childhood) was established.

He was then given sulphapyridine ,2 g daily, with immediate benefit. No new vesicles appeared after the 3rd day of treatment and his joint pains settled completely within a week. He remained well on a dose of 250 mg/day over the next 2 months. The tablets were then stopped and within a week the vesicles and the joint symptoms reappeared, both resolving again on renewing treatment. After a further 12 months of treatment the drug was successfully withdrawn. No skin lesions or joint pains were noted 15 months after treatment had been discontinued.

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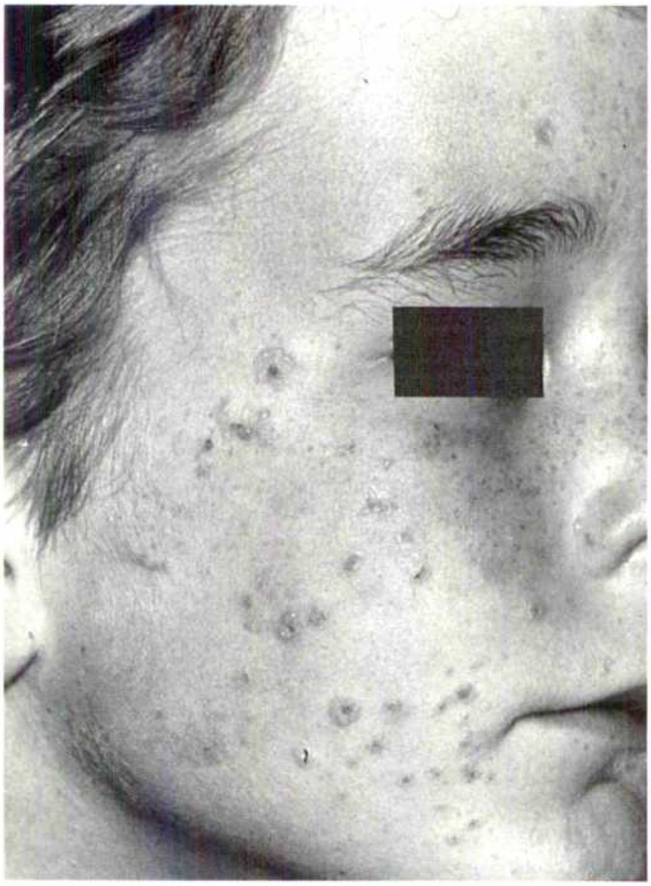

FIGURE 1. Patient 1. Facial vesicles seen in the 17th week of his illness.

Investigations. During the first 5 weeks the patient's haemoglobin dropped to 9·4 g/dl with normal indices, but had returned to normal by the 3rd month. The white cell count was normal, the ESR was 130 mm/h initially and slowly fell to 40 mm/h by the 4th month. The ASO titre was greater than 800 U/ml in the 5th and 8th weeks, and on the second occasion the AHT was 1024 IU/ml. Other investigations for an infective cause were all negative initially, including viral cultures of skin lesions, blood cultures and a battery of viral antibody tests. Brucella, salmonella, shigella, yersinia, Q-fever and mycoplasma infections were excluded. However, in the 8th week a throat swab grew a beta-haemolytic streptococcus Lancefield group A. Immunoglobulins, serum creatinine phosphokinase, serum B12 and red cell folate were all in the normal range and ANF, DNA binding, crithidia, RA latex, urinary aminoacids, Paul Bunnell, urinary porphyrins and Coomb's tests were also negative.

Immunohistopathology. A biopsy of a vesicle from the left arm showed a split at the dermo-epidermal junction with exocytosis of neutrophil polymorphs into the epidermis. Small collections of neutrophils were also present in the superficial dermis. There was a scanty perivascular lymphocytic infiltrate.

Skin taken from the buttock for direct immunofluorescence showed a linear band of IgA at the dermo-epidermal junction. Circulating anti-basement membrane zone antibodies were not detected by indirect immunofluorescence.

Patient 2

A 34-year-old Asian woman suffered an upper respiratory tract infection with fever and a sore throat. Ten days later she developed severe joint pains in her hands, wrists, ankles and knees accompanied by an itchy vesicular eruption on her chin, trunk, buttocks, elbows, knees and ankles. On examination in the 4th week of her illness there were annular lesions with papules and vesicles at all of these sites including periorally. The oral mucous membrane was not involved and there was no joint swelling. A diagnosis of linear IgA dermatosis was made.

She was treated with 30 mg prednisolone daily with some improvement. Dapsone 100 mg daily was then added and both the eruption and her joint symptoms cleared rapidly.

Histopathology. Histology showed a sub-epidermal bulla and direct immunofluorescence showed linear IgA deposition at the dermo-epidermal junction. No circulating antibody was demonstrated.

DISCUSSION

Recent studies have suggested that linear IgA dermatosis of childhood (chronic bullous dermatosis of childhood) and linear IgA dermatosis of adults are simply different expressions of the same disease.[1,2]

Our two patients, one an adult and the other a child, developed linear IgA dermatosis some 8 to 10 days after a fever and sore throat. In the adult the symptoms were attributed to a viral illness, but in the child, who was extensively investigated, there was evidence of a streptococcal throat infection (raised ASO titre 5 and 8 weeks after onset of symptoms). It is of interest that prior to his illness he had been bottle feeding lambs, two of which had recently suffered from 'joint ill' a suppurative arthritis. Several organisms have been linked with 'joint ill' including streptococci of Lancefield groups C and D.[3] However, it was a Lancefield group A streptococcus that was actually isolated from the patient's throat, albeit in the 8th week of his illness. In our experience of 29 children with linear IgA dermatosis, the eruption was preceded by an upper respiratory tract infection or a viral exanthem, such as chicken pox, in 11 cases.[2] Six of 24 adult patients also had a preceding infection ranging in severity from an upper respiratory tract infection to brucellosis and tetanus. Many of the children had received antibiotics and 20% of the adults had taken non-steroidal anti-inflammatory drugs prior to the onset of their eruption.

In a series of 22 children with childhood dermatitis herpetiformis only five had systemic symptoms.[4] Two patients lost weight, one had malaise, one had a low grade fever and one anorexia. A mild secondary anaemia was also present in one case. Transient pain and stiffness in the hands and feet has been described in a child with linear IgA dermatosis,[5] but severe arthralgia of the type found in our patients is exceptional and has not been reported before to our knowledge. Indeed the symptoms of arthralgia in Patient 1, together with the high ESR and anaemia, dominated the clinical picture and led to considerable diagnostic difficulty. In both cases, treatment aimed at the bullous eruption, i.e, sulphapyridine and dapsone resulted in dramatic improvement of the joint pain as well as the skin.

ACKNOWLEDGMENTS

Patient 1 was referred to St. George's Hospital by Dr P.R.Coburn, Consultant Dermatologist, and Dr A.Robinson, Consultant Paediatrician, St Richard's Hospital, Chichester. Patient 2 is under the care of Dr J.S.Pasricha, All India Institute of Medical Sciences, New Delhi. Veterinary advice was provided by Mr M.L.Mair, BVMS, MRCVS.

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Ectopic cutaneous schistosomiasis and schistosomal ocular inflammatory disease

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SUMMARY

We describe a patient with unilateral ectopic cutaneous schistosomiasis as a feature of *Schistosoma mansoni* infection. In addition to skin lesions he also suffered from schistosomal ocular inflammatory disease. The infection appeared to have been acquired during a visit to Tanzania. Ectopic cutaneous lesions usually affect the perineal area or trunk, and our patient is unusual in the localization of his skin lesions to the forehead.

CASE REPORT

A 17-year-old Tanzanian man attended the Eye Casualty Department of the Leicester Royal Infirmary complaining of blurring of vision in his right eye of 2 weeks duration. Fundoscopy revealed macular exudates, choroiditis, and areas of focal change in the retinal pigmentary epithelium of the right eye. The ocular aspects of this case are unusual, and will be reported separately in the ophthalmology literature. He also complained of a slightly itchy eruption of his forehead of similar duration. He was otherwise perfectly well.

He was resident in Leicester, but had returned from a visit to relatives in Tanzania approximately 4 weeks prior to the onset of his symptoms. He visited Tanzania annually, and during his recent stay he had been swimming in Lake Victoria, and recollected a short febrile illness and an episode of diarrhoea shortly thereafter.

Because it was considered that there might be a connection between his skin lesions and his ocular problem he was referred to the Dermatology Department. Examination revealed a group of papules on the left side of the forehead (Fig. 1). A skin biopsy was taken from this area.

Histology showed an abscess containing numerous eosinophils centred on a hair follicle. Further sections revealed two schistosome ova (Fig. 2). A full blood count showed a 47% eosinophilia (WBC $5{\cdot}31 \times 10^9$/l). Urine examination for ova was negative. Stool examination (salt flotation method) revealed ova of *Schistosoma mansoni*. The enzyme-linked immunosorbent assay (ELISA) for *S. mansoni* ovum antigen was positive.

The patient was treated with a single dose of praziquantel (40 mg/kg body weight). His skin

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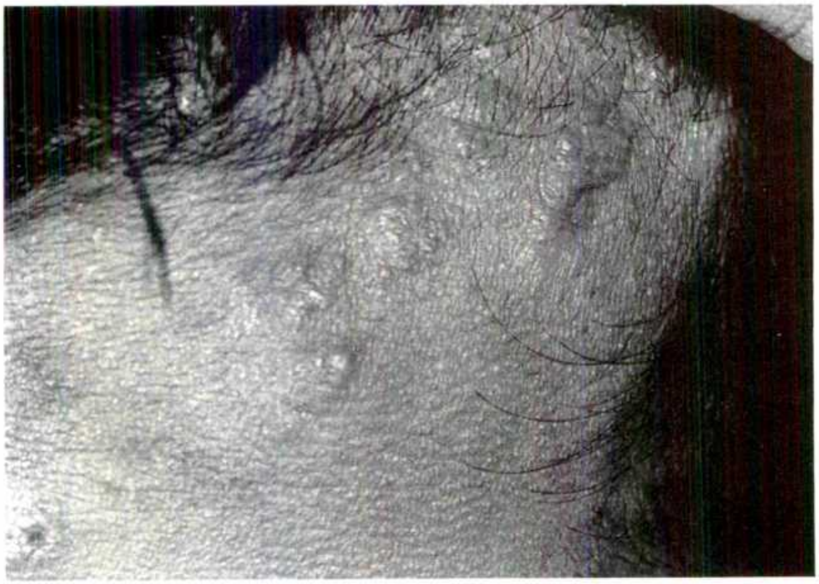

FIGURE 1. Group of papules on the left side of the forehead.

and ocular lesions had resolved 3 months later. Subsequent stool examinations did not reveal any schistosome ova.

DISCUSSION

Flukes of the family Schistosomatidae (phylum Platyhelminthes, class Trematoda) are of enormous medical importance. Three major species of schistosomes infect man—*Schistosoma haematobium*, *S. japonicum* and *S. mansoni*. It has been estimated that worldwide possibly 200,000,000 people are infected, and 500,000,000 are exposed to the threat of infection.[1]

Schistosomiasis caused by *S. haematobium* is widespread in Africa and the Arabian peninsula, and *S. japonicum* infection is found in the Far East, parts of mainland China, Japan, Taiwan, the Philippines, Indonesia, Thailand, Laos, Cambodia and Malaysia. *S. mansoni* infection was originally an African disease, but was imported into the West Indies and South America by the slave trade. It occurs commonly in tropical Africa, the Arabian peninsula, Brazil, Venezuala and certain Caribbean islands.

Adult schistosomes live in the veins which drain certain of the host's viscera, and each species has a particular preference for its location. *S. haematobium* lives principally in the peri-vesical venous plexus; *S. japonicum* in tributaries of the superior mesenteric vein draining the small intestine; *S. mansoni* prefers tributaries of the inferior mesenteric vein draining the colon. Eggs released by the adult female flukes penetrate the wall of the adjacent viscus and are expelled in the urine (*S. haematobium*) or the faeces (*S. japonicum* and *S. mansoni*). Eggs may also be carried in the portal blood to the liver, via portal-systemic anastomoses to the inferior vena cava and eventually the lungs, and in the case of *S. haematobium* directly into the vena cava via the internal iliac veins. The eggs of each species differ in morphology—*S. haematobium* eggs have a terminal spine, those of *S. mansoni* a lateral spine, and *S. japonicum* eggs are devoid of spines. When these eggs in human excreta are deposited in water they hatch, each egg releasing a free-swimming

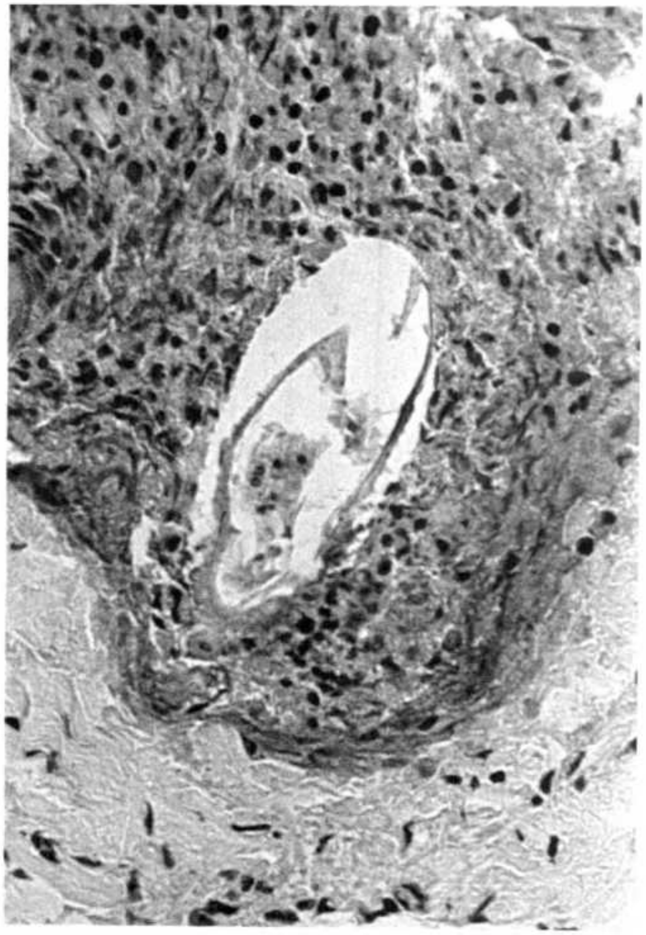

FIGURE 2. Schistosome ovum in the dermis (Original × 400).

embryo, the miracidium. Miracidia penetrate certain species of fresh water snails which are the intermediate hosts of these parasites. Within a snail each miracidium develops into a sporocyst, which subsequently gives rise to numerous daughter sporocysts. The daughter sporocysts produce several thousand fork-tailed cercariae which escape from the snail into the water. If man strays into water containing cercariae they rapidly penetrate the skin, losing their tails in the process. Entering the peripheral circulation the schistosomula, as they are now called, are carried to the heart and then via pulmonary capillaries to the arterial system. Only schistosomula which reach the mesenteric arteries, traverse the intestinal capillary bed, and reach the liver via the portal system, can continue to develop. Those which are carried into other arterial channels do not survive. After a period of approximately 3 weeks development in the liver sinusoids, the adult flukes migrate against the portal blood flow to the walls of the gut or bladder (*S. haematobium* adults reach the vesical plexus after migrating from the inferior mesenteric vein via the rectal venous plexus), pair, and begin egg production.

The clinicopathological features of schistosomiasis can be related to different parts of the schistosome life-cycle.[1-3] Cercarial penetration of the skin may be asymptomatic, or produce a transient pruritic dermatosis (schistosomal dermatitis).

Maturation of schistosomes, migration to their preferred locaton in gut or bladder, and commencement of egg-laying, may produce a febrile illness with malaise, arthralgia, headache, cough, pruritus, urticaria and angioedema. In *S. japonicum* infection this stage has been referred to as the Katayama syndrome, and it is usually accompanied by a pronounced blood eosinophilia.

The passage of schistosome eggs through the walls of viscera is accompanied by an inflammatory reaction and granuloma formation. In urinary schistosomiasis this stage is marked by haematuria, dysuria and frequency. In intestinal schistosomiasis there may be abdominal discomfort and episodic diarrhoea. Large numbers of eggs are present in the excreta.

The later stages of infection are marked by progressive fibrosis around pre-existing granulomata, with a marked reduction in egg excretion. Fibrosis around eggs in the lungs may lead to pulmonary hypertension and cor pulmonale, and hepatic fibrosis to hepatosplenomegaly, portal hypertension and ascites. In urinary schistosomiasis the contracted bladder is responsible for urinary frequency, and carcinoma of the bladder may be a late sequel.

In addition to the occurrence of schistosomal dermatitis and urticarial reactions in the early stages of the disease, an established infection may be accompanied by paragenital granulomata and fistula formation, and ectopic cutaneous schistosomiasis.[4] In endemic areas, schistosomal granulomata of the perineum and genitalia are not uncommon.[5-8]

Paragenital lesions are usually papular initially, but gradually enlarge into warty, vegetating masses. Extensive fistulous tracts may develop in the perineum and buttocks, and carcinoma arising in paragenital schistosomiasis has been reported.[5] It is not surprising, in view of the communications between the peri-vesical, prostatic, vaginal and rectal venous plexuses, that adults and eggs of *S. haematobium* occasionally find their way into the veins of the perineum and produce granulomas. *S. mansoni* flukes and eggs may also find their way into the perineal skin,[7] presumably via the communication between the inferior mesenteric vein and the rectal venous plexus.

Extragenital ectopic cutaneous localisation of ova is also a well-documented manifestation of schistosomiasis. In most reported cases pruritic papular lesions have occurred on the trunk, and although occasional reports describe extensive skin lesions,[9] and others mention individuals in whom the lesions were localized to the peri-umbilical area,[10,11] a substantial number provide clinical descriptions suggesting a zosteriform distribution of the eruption.[12-20] The majority of these cases with lesions in a zosteriform distribution have been patients with *S. haematobium* infection. Jacyk, Lawande and Tulpule[21] state that a zosteriform pattern of lesions has not been observed in individuals infected with *S. mansoni*, but the clinical descriptions of cases in some reports of ectopic mansoni schistosomiasis certainly suggest this pattern of lesions.[13,14,16] The occurrence of ectopic cutaneous schistosomiasis affecting the head and neck area would appear to be a very rare phenomonon.[21,22]

In addition to cutaneous lesions, ectopic localization of schistosomes may involve the central nervous system,[13,15,23-27] the gall bladder,[28] the female reproductive organs,[29,30] the eyes,[31-35] and the ocular adnexae.[36] Oréfice, Simal and Pittella[34] described the fundoscopic changes seen in schistosomal choroiditis, and Pittella and Oréfice[35] reported the first histologically confirmed case of schistosomal choroiditis produced by *S. mansoni*.

There has been a great deal of speculation in the literature, by many authors, attempting to explain how viable schistosome eggs reach ectopic locations. This problem was reviewed by Faust[37] who discussed the theories which might account for this phenomenon. These include: (i) Cercariae enter the skin or mucous membranes and develop directly into adults in nearby veins, without the necessity of an incubation stage in the liver. Faust[37] cites several reports which confirm that develoment in the intrahepatic portal veins is essential to the parasite, and this theory of local development is therefore not tenable. A local route of infection does not appear to be responsible for ectopic ocular schistosomiasis.[38] (ii) A patent foramen ovale would allow eggs to pass directly from the venous to the arterial circulation, and subsequently be widely disseminated. A patent foramen ovale was discovered at autopsy in the case reported by Pittella and Oréfice,[35] but the likelihood of this abnormality being responsible for more than an occasional case of ectopic schistosomiasis seems extremely remote. (iii) Eggs deposited by flukes in their usual locations traverse the capillary barriers in the liver, and possibly natural or pathological arteriovenous fistulae in the lungs,[39] to reach the general circulation. If this occurred, the tendency would be for eggs to be widely disseminated, whereas in many cases they occur in localized clumps in ectopic foci. (iv) Adult worms migrate out of their normal habitats, and travel to distant sites where they deposit eggs in venules. MacDonald and Morrison[11] suggested that the peri-umbilical location of skin lesions in their cases might be explained by parasites reaching epigastric veins via the para-umbilical veins in the ligamentum teres. Although this theory appears to provide a plausible explanation for the clumping of ova in ectopic sites, and the zosteriform distribution of skin lesions, adult flukes have only rarely been demonstrated in ectopic locations.[27,31,32] (v) Adults or eggs may reach ectopic sites via the vertebral venous system, which communicates with the veins of the body cavities.[40] Raised intra-abdominal and intra-thoracic pressure during straining or coughing squeezes blood out of the thoraco-abdominal cavity into the vertebral venous system, and could explain dissemination of adult flukes or clumps of eggs.

It has not been established with certainty which of these suggested explanations for ectopic schistosomiasis is valid, but (iv) and (v) would appear to be the most plausible. However, in cases such as ours, where the ectopic lesions are distant from the usual habitat of the parasite, and where they are so localized, it appears unlikely to us that embolization of eggs by a purely venous route can explain the ectopic lesions. The migration of paired adult flukes via a venous route to these areas would be an incredibly tortuous journey against the blood flow, and also appears unlikely, although the localization of lesions would certainly be more consistent with production of eggs by adults in the vicinity. At present there does not appear to be a solution to this intriguing puzzle.

In anyone returning from an endemic area, it is important to consider the possibility of ectopic cutaneous schistosomiasis in the differential diagnosis of pruritic, papular skin lesions, particularly if these have a peri-umbilical or zosteriform distribution on the chest or abdominal skin.

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Epidermal antigens in pemphigus vegetans. Report of a case

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SUMMARY

A patient is described who had typical pemphigus vegetans, according to clinical, histopathological and immunofluorescence criteria. To determine the specific molecules bound by antibodies in the serum of this patient, an immunoprecipitation analysis was performed with extracts of [^{14}C]-amino acid labelled cultured normal human keratinocytes. The patient's serum precipitated the 130 kd and 85 kd polypeptides of the pemphigus vulgaris antigen, but also precipitated other polypeptides, the significance of which is not clear.

Pemphigus vegetans is a distinct variety of pemphigus vulgaris. It differs from typical pemphigus vulgaris both clinically, in that primarily the flexures are affected with vegetating erosions, and histologically, in that biopsies reveal considerable acanthosis with papillomatosis and intra-epidermal abscesses containing eosinophils, while acantholysis may not be apparent.[1] In spite of these clinical and histological differences, direct and indirect immunofluorescence results are the same in typical pemphigus vulgaris and in pemphigus vegetans.

We describe here a patient with pemphigus vegetans, and present direct and indirect immunofluorescence results. We investigated the antibody binding specificity of the patient's serum, in comparison to that of pemphigus vulgaris serum using an immunoprecipitation technique.

CASE REPORT

A 55-year-old woman was referred to us with a 5-month history of progressive skin disease. In January 1986 she developed vesico-bullous lesions on the previously normal skin of both axillae, and the submammary and intragluteal folds. Treatment with topical steroids was successful, but 2 months later oral erosions and flexural vegetating plaques developed and required treatment with oral prednisone. Although the skin lesions improved, the treatment had to be discontinued because of the occurrence of steroid psychosis.

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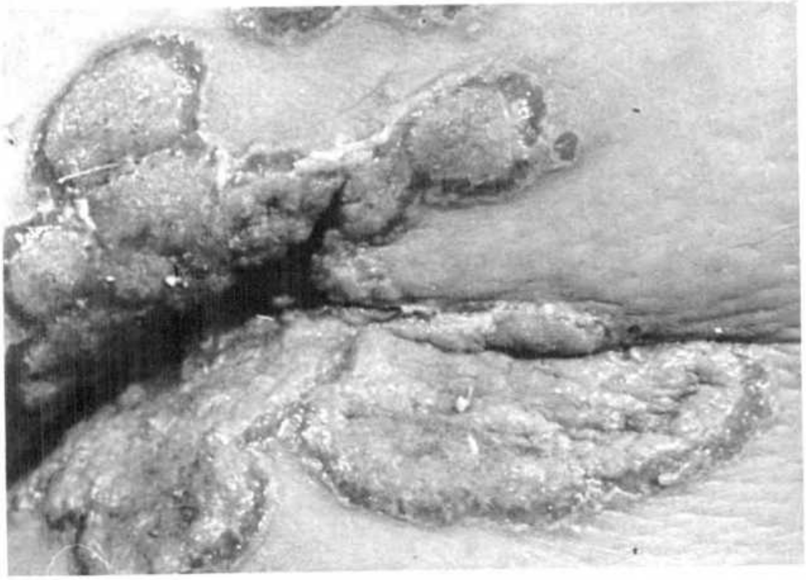

FIGURE 1. Erosive and vegetating lesions in the inguinal flexure.

On examination in May 1986, she exhibited thick, vegetating, malodorous, violaceous plaques with eroded borders in the axillary, submammary, inguinal, umbilical and popliteal folds (Fig. 1). Florid vegetations were also present on the back and thighs. There were erosions on the palate, tongue and lips.

Routine laboratory tests were normal with the exception of ESR 45 mm/h, WBC 13·32 × 10^9/l with 22·5% eosinophils (3·1 × 10^9/l) and platelets 0·501 × 10^{12}/l.

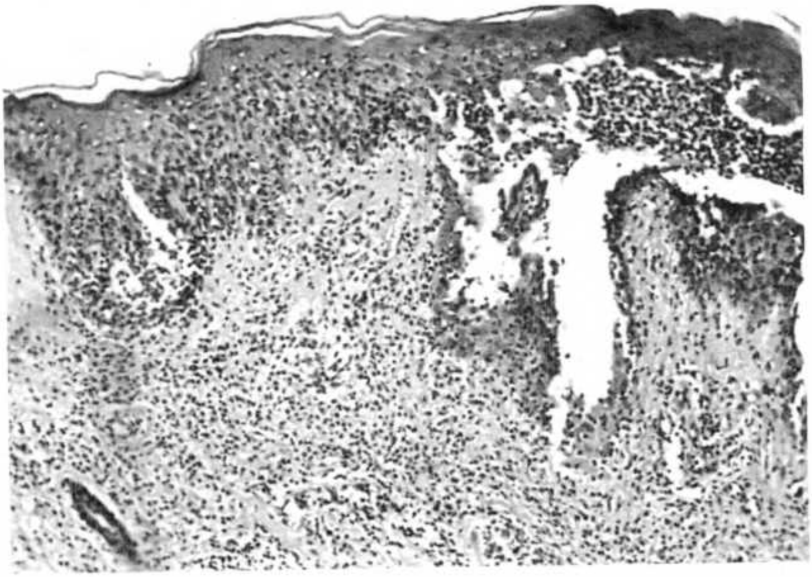

FIGURE 2. Intraepidermal clefts with acantholysis and eosinophils (Original × 100)

Histopathology of an early lesion revealed papillomatosis with acanthosis and suprabasal clefts with acantholysis (Fig. 2). Eosinophils were abundant throughout the dermis and epidermis, and formed intra-epidermal abscesses. Direct immunofluorescence showed deposits of IgG and C_3 in the intercellular spaces in the epidermis. Indirect immunofluorescence with FITC staining confirmed the presence of numerous eosinophils. Indirect immunofluorescence on monkey eosophagus demonstrated anti-intercellular antibodies (IgG) at a final titre of 1:1280.

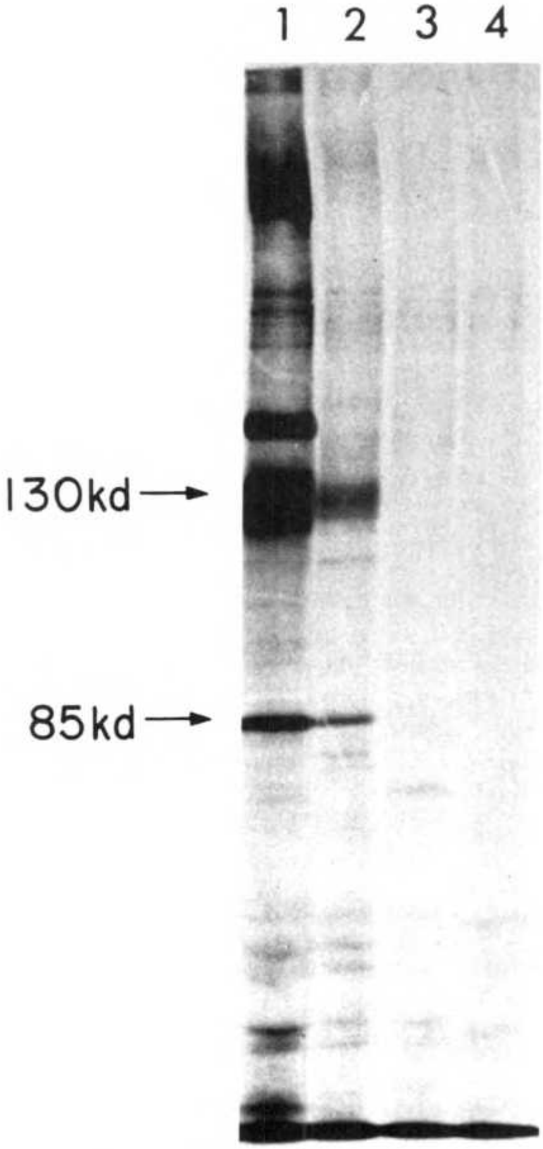

FIGURE 3. Immunoprecipitation results. Lane 1: serum from our patient. Lane 2: pemphigus vulgaris control serum. Lanes 3 and 4: normal human control sera. Arrows indicate the 130 kd and 85 kd polypeptides of the pemphigus vulgaris antigen.

The antigenic specificity of the patient's serum was then studied by an immunoprecipitation technique on extracts of [^{14}C]-amino acid labelled cultured normal human keratinocytes.[2] The serum precipitated polypeptides that have been shown previously to be specific for pemphigus vulgaris, namely chains of molecular weight 130 and 85 kd, but also precipitated some other chains that did not correspond to those precipitated by normal sera or serum from typical pemphigus vulgaris (Fig. 3).

Treatment with cyclophosphamide (150 mg/day) and eight sessions of plasmapheresis, initiated in order to avoid any relapse of the steroid psychosis, did not improve the patient's condition. Subsequent treatment with 1 mg/kg/day Deflazacort (2′-methyl,16,17-oxazolin prednisolone acetate) cleared the lesions in a few weeks.

DISCUSSION

There is little doubt that our patient had pemphigus vegetans. Classically, two types of pemphigus vegetans have been described, the Neumann type, begining as erosions which later vegetate, and the Hallopeau type, beginning with pustules. The clinical differences are associated with diverse histopathological features, the Neumann type showing intraepidermal vesicles with suprabasal acantholysis and the Hallopeau type exhibiting intraepidermal abscesses containing eosinophils, but with only occasional acantholysis.[3]

Our patient belongs clinically to the Neumann type, though a mixture of the histopathological features of both types were present.

In recent years attempts have been made to characterize the various forms of pemphigus by isolating antigens specific to each.[2,4,5] In particular, Stanley *et al.*[2] have shown by immunoprecipitation analysis with extracts of mouse and normal human keratinocytes, that sera from patients with pemphigus vulgaris specifically precipitated a glycoprotein from the keratinocyte surface with disulphide-linked chains of 130 and 85 kd. This pemphigus vulgaris antigen glycoprotein was shown to differ from the 160 kd molecule that binds antibodies from patients with pemphigus foliaceous.

Serum from our patient contained antibodies that precipitated the 130 and 85 kd polypeptides of the pemphigus vulgaris antigen, but also precipitated some other chains, the significance of which is not clear at present. It may be, therefore, that pemphigus vegetans belongs to the same group as pemphigus vulgaris, but differs in having, in addition to the antibodies typical of pemphigus vulgaris, antibodies directed against some other, as yet uncharacterized, antigens.

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Subcorneal pustular dermatosis (Sneddon-Wilkinson disease) with amicrobial lymph node suppuration and aseptic spleen abscesses

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SUMMARY

Subcorneal pustular dermatosis (SPD) or Sneddon-Wilkinson disease is a skin eruption in which concomitant systemic involvement has not been reported to our knowledge. We describe a patient suffering from SPD of 6 years duration with amicrobial inguinal lymph node suppuration and aseptic spleen abscesses followed by pyoderma gangrenosum.

CASE REPORT

A 39-year-old woman was admitted to hospital in February 1981 with a fever of 14 days duration and bilateral swelling of the inguinal lymph nodes. Six days of tetracycline treatment (2 g daily) had been ineffective. On physical examination she had a temperature of 38·8°C, erythematous skin in the inguinal area, with bilateral 5 cm diameter painful lymph nodes. Pustules surrounded by erythematous areas were observed on the trunk. There was no hepatomegaly or splenomegaly, and no evidence of leg, skin or genital infection was found. The white blood cell count was $14{\cdot}5 \times 10^9$/l and the ESR was 86 mm. Puncture of inguinal nodes revealed altered neutrophils. However, pus cultures for *Haemophilus ducreyi* and usual bacteria were negative. Blood, urine and pustule cultures were sterile. Pristinamicin and gentamicin were given without improvement of the inguinal node swelling. The skin pustules dried up within two days. Surgical drainage of the lymph nodes and treatment with trimethoprim-sulphamethoxazole led to the patient becoming apyrexial within 1 week. Cultures for mycobacteria, bacteria and fungi failed to demonstrate an infectious process. A chlamydial group complement fixation test was negative. The patient was discharged.

In April 1981, pustules reappeared on the trunk, and arms without systemic symptoms. The pustules were surrounded by erythematous maculae; they persisted for 1 day then ruptured and dried, forming leafy scales. The transient pustular eruption recurred every 2 or 3 months. Several biopsies of old scaly lesions were not informative.

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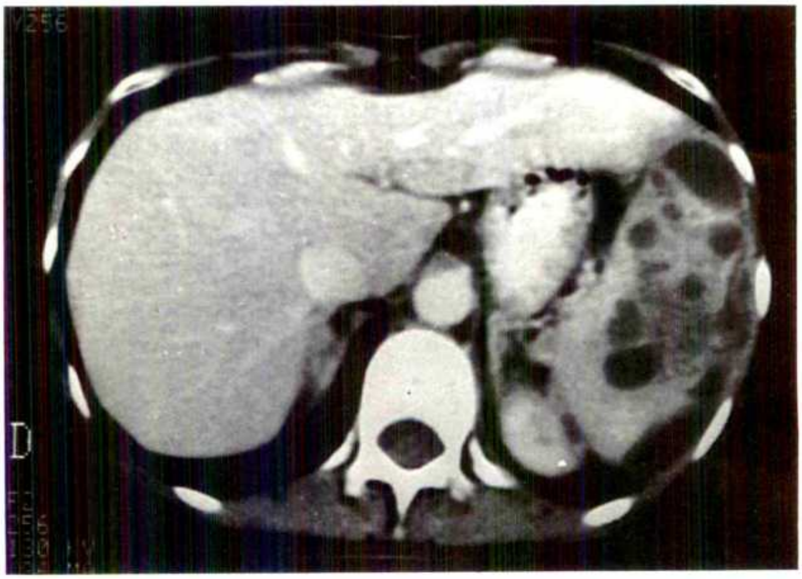

FIGURE 1. Computerized axial tomography showing spleen defects.

In July 1986, the patient was admitted to hospital with a fever of 1 week duration (38°C). Physical examination revealed splenomegaly without hepatomegaly or lymphadenopathy. The white blood cell count was $16{\cdot}0 \times 10^9$/l with 80% neutrophils; the ESR was 36 mm. Blood cultures were sterile. Computerized axial tomography revealed multiple defects in the spleen (Fig. 1). Splenectomy was performed. Cavities containing pus were found in the lower part of the spleen. Pathological examination of the spleen showed polymorphonuclear leukocyte infiltration forming suppurative areas. There was neither tumour tissue nor vascular changes. Cultures of spleen tissue and pus failed to demonstrate any infectious process (bacterial, mycobacterial or fungal). Within 10 days, the patient was apyrexial. The white blood cell count was $10{\cdot}0 \times 10^9$/l. The patient was discharged.

In August 1986, the patient was referred to us because pustules had appeared on the splenectomy scar. The lesions showed peripheral spreading with central healing (Fig. 2) tending to be grouped in a gyrate arrangement). In some pustules, pus had accumulated in the lower half. Isolated transient pustules were still appearing on the trunk.

Biopsy of an isolated pustule showed a subcorneal bulla containing polymorphonuclear leukocytes with no acantholysis (Fig. 3). Direct and indirect immunofluorescence tests, using standard techniques, did not reveal any epidermal deposits. Cultures from the pustules grew no organisms. Serum immunoelectrophoresis revealed the presence of a homogenous protein IgA with kappa light chains. The immunoglobulin was absent from the urine. X-rays revealed no bone lesions. A bone marrow biopsy showed no infiltration of plasma cells. Tests of polymorphonuclear leukocyte function, including chemotaxis and intracellular killing were normal. The patient was given dapsone (100 mg daily) which produced complete clinical clearance, of both the annular pustular eruption and the isolated pustules within 2 weeks. After 4 months in complete remission on dapsone (100 mg daily), the therapy was progressively decreased to a 25 mg daily dose. Transient pustules then recurred and a 3 cm bullous lesion on an erythematous base appeared on the left leg without systemic symptoms. Two weeks of dapsone

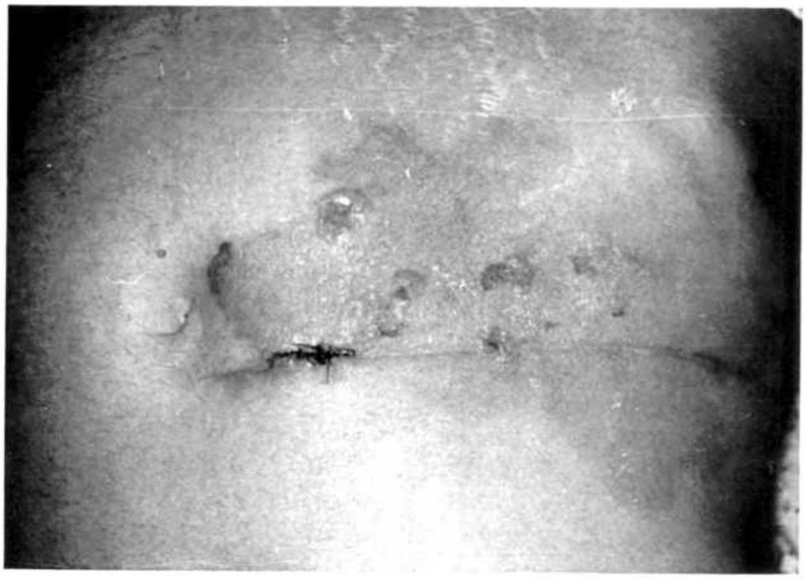

FIGURE 2. Gyrate eruption on the splenectomy scar.

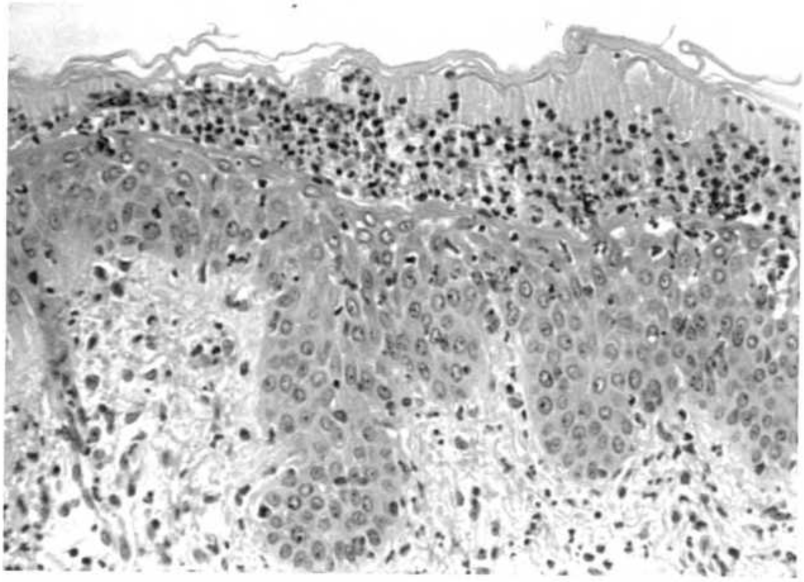

FIGURE 3. Subcorneal pustule (Haematoxylin and eosin original × 100).

therapy (100 mg daily) were partially effective; the transient pustules disappeared, but the bulla on the left leg became larger, ruptured and formed a 10 cm ulcer. The lesion had an elevated red border and a base with small abscesses. A halo of bright erythema surrounded the edge of the ulcer. This ulceration was characteristic of pyoderma gangrenosum. Two months of systemic corticosteroid therapy (1·5 mg/kg/day) led to the complete resolution of the pustular eruption and the pyoderma gangrenosum.

DISCUSSION

The patient presented with a chronic, relapsing, pustular eruption mainly involving the trunk. The eruption tended to coalesce and form a circinate pattern. Histologically, the lesions showed a subcorneal bulla containing neutrophils. Complete remission was obtained with dapsone therapy. These findings are consistent with a diagnosis of subcorneal pustular dematosis (SPD) as first described by Sneddon and Wilkinson.[1,2]

The patient had SPD associated with IgA paraproteinaemia and pyoderma gangrenosum without myeloma. IgA paraproteinaemia has been detected in a few patients with SPD.[3–7] Direct immunofluorescence studies on the skin occasionally revealed subcorneal or epidermal intercellular IgA deposits.[4–6,8] Our patient had no epidermal IgA deposits. SPD has been reported to be associated with IgA paraproteinemia and pyoderma gangrenosum in only three cases to our knowledge.[3,7,9]

SPD is known to be a clinical manifestation of a wide spectrum of neutrophilic dermatoses including in particular pyoderma gangrenosum and Sweet's syndrome.[10] In these dermatoses, histological examination reveals mature polymorphonuclear leukocytes within the epidermis or the dermis or both. These leukocytes are not neoplastic and infecting organisms are never isolated. The pathogenesis of the neutrophilic dermatoses is still uncertain. Abnormalities of neutrophil function have occasionally been reported. However, consistent patterns have not emerged. In our patient, neutrophil chemotaxis and intracellular killing were normal. Occasional association of neutrophilic dermatosis with immunological diseases, such as immunoglobulin paraproteinemia in SPD or pyoderma gangrenosum,[11] may be more than a mere coincidence and suggests that immunological mechanisms may be involved. Systemic involvement has been reported in Sweet's syndrome only, Matta *et al.*[12] described a patient with Sweet's syndrome, and neutrophilic infiltration of the hepatic parenchyma. Gibson *et al.*[13] reported the case of an agnogenic myeloid metaplasia associated with Sweet's syndrome and a dense aseptic polymorphonuclear leukocyte aseptic infiltration of the lung.

This is the first report to our knowledge of SPD with amicrobial suppurative inguinal lymph nodes and aseptic spleen abscesses. These associations suggest that polymorphonuclear leukocyte infiltration of internal organs may occur in SPD.

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News and Notices

Seventh Course for Consultants and Senior Registrars, 1989
Institute of Dermatology

With the generous support of Sandoz (U.K.) a course will be held on Autoimmune Connective Tissue Diseases: Recent Advances on the 17–19 February 1989 at the Metropole Hotel, Brighton. The faculty will be an international one and the programme will include workshops and lectures on wide aspects of pathophysiology, diagnosis and treatment. For further details please contact the course director, Professor M.W. Greaves or Miss Angela Dell, at the Institute of Dermatology, St. Thomas's Hospital, Lambeth Palace Road, London SE1 7EH, U.K. Telephone: 01-928-9292 Ext. 3314.

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